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THE SHEATH OF NEUMANN

Experimental Proof of Double Staining in Circumtubular Areas of Dentin

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Dentin, which forms the bulk of a tooth, is a tubular calcified connective-tissue, transversed by minute canals radiating from the cavum pulpae toward the periphery of the tooth. These canals, termed dentinal tubuli, contain cytoplasmic extensions of certain peripheral cells of the pulp: the odontoblasts or fibrilloblasts. These extensions have received the name of dentinal fibrils. So small are the diameters of these tubules that, to the earliest investigators (1, 2), they seemed to be fibers. The first description of dentin that mentioned its tubular nature was made by van Leeuwenhoek (3). Fränkel (4), Retzius (5), and Purkinje (6), observed circular layers around the dentinal tubules. It does not seem possible to determine whether the layers noted by these investigators are comparable with what is now called a sheath. In 1854, Kölliker (7) first isolated dentinal tubules. In 1863, Neumann (8) stated that dentinal tubuli are surrounded by definite walls, which he called *Zahnscheiden:* dental sheaths. These sheaths have been named sheaths of Neumann. Henle (9), Magitôt (10), and Sudduth (11), denied the presence of such a sheath. Höhl (12) affirmed its existence, but failed to find the sheath in the dento-genetic zone. Römer (13) denied its presence; Underwood (14) expressed doubt as to its existence. From 1905 to almost the present day, textbooks on dental histology describe a "sheath of Neumann." Fleischmann (15), Tomes (16), Hopewell-Smith (17), and others, adhered to the idea of a definite sheath surrounding the lumena of dentinal tubuli. Hanazawa (18), in his exhaustive study of dentin, stated that the tubular wall cannot be isolated. He described an area around the lumen of the dentinal

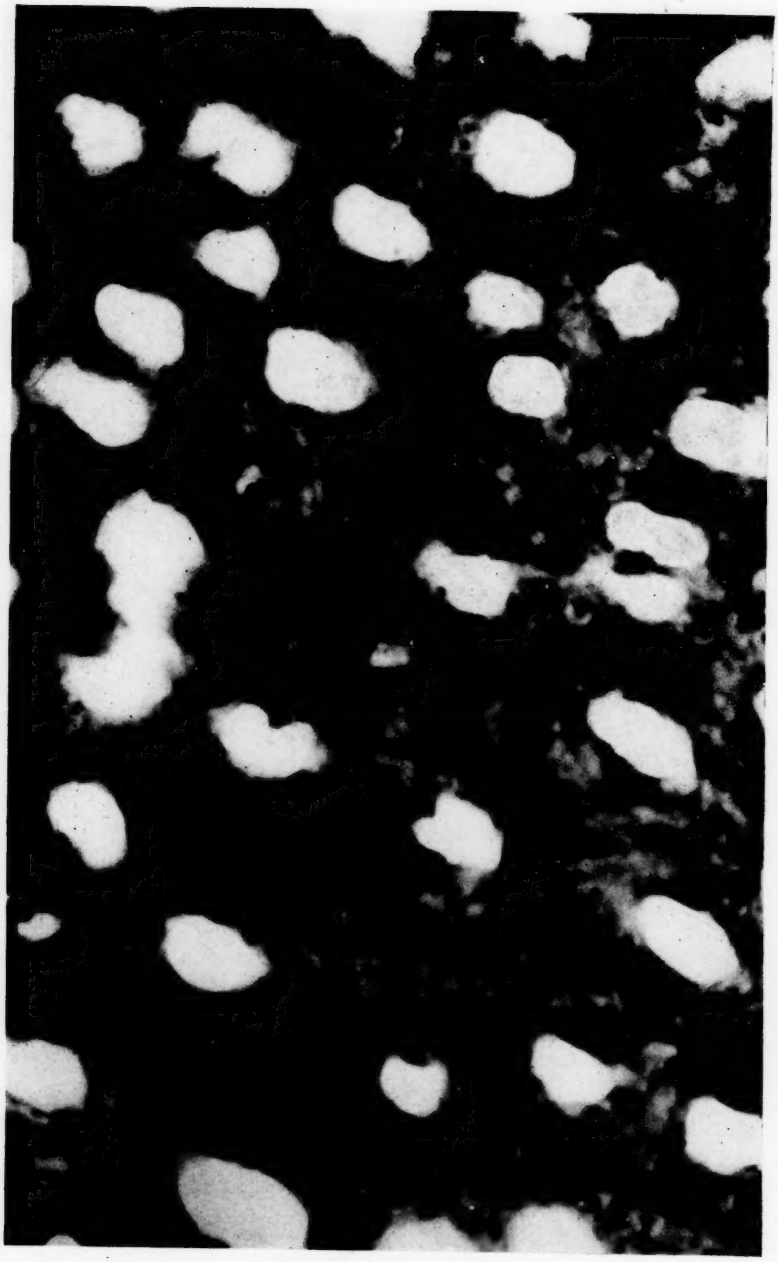

Fig. 1. Transverse section of human dentin. × 6000 ±

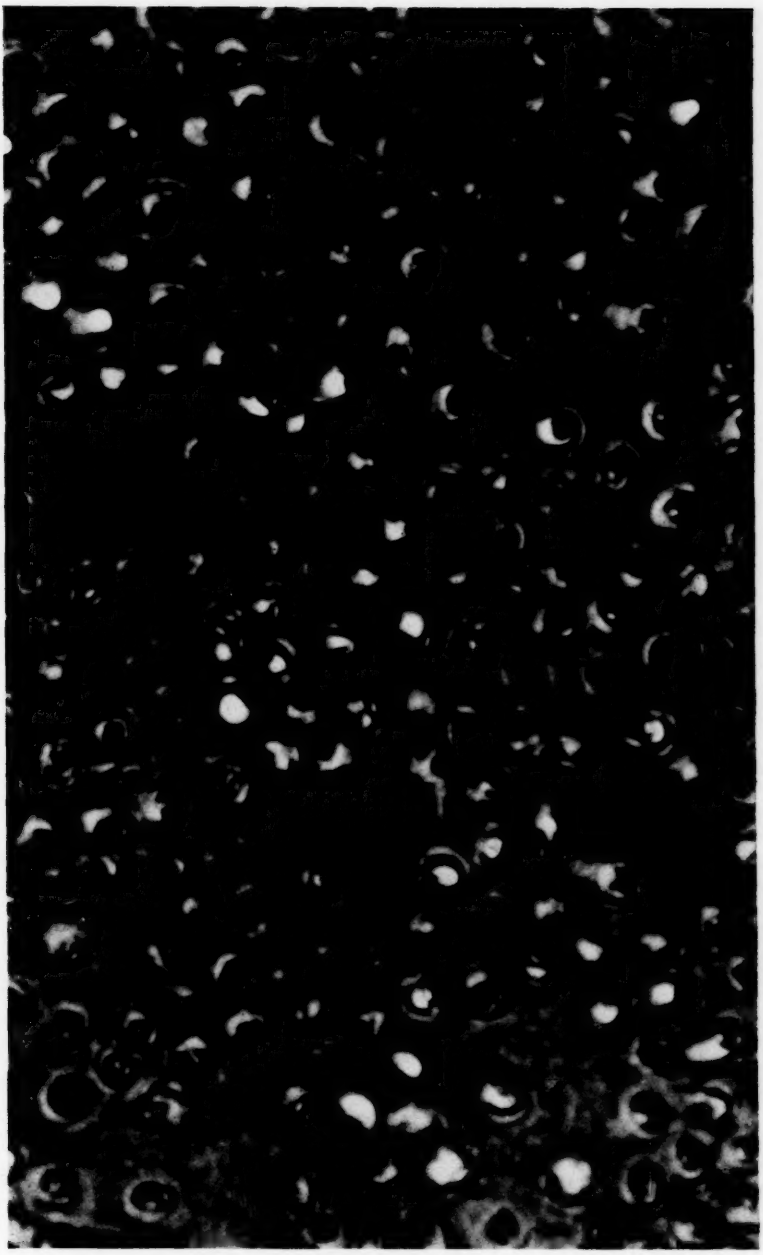

Fig. 2. Transverse section of human dentin. × 2000 ±

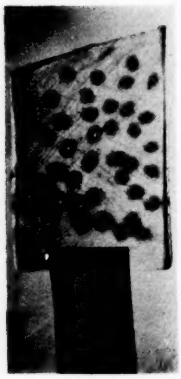

Fig. 3

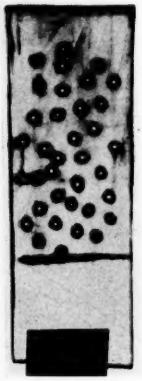

Fig. 4

Fig. 5

Figs. 3, 4 and 5. Sections of perforated celloidin (thickness, 3 mm.) stained with hematoxylin and eosin (*fig. 3*), hematoxylin (*fig. 4*), and Mallory triple connective-tissue stain (*fig. 5*). Although the celloidin is perfectly homogeneous, darkly stained areas are produced around the perforations.

Fig. 6

Fig. 7

Fig. 6. Photomicrograph of unstained dentinal fibrils, in *diffuse* light (Meyer). × 1000 ±.

Fig. 7. Photomicrograph of section in *fig. 6*, in *direct* light (Meyer). Through diffraction an artefact is created simulating sheaths of Neumann. × 1000 ±.

tubule that was "slightly calcified or uncalcified," but believed it "unreasonable to call this area a sheath." On the other hand, Bödecker (19) ascribes a definite function to this structure: on page 206 of his Dental Histology and Embryology he states that "the function of the sheath cannot be to limit the fluids to the tubes, for this is already enclosed in the latter. The only logical deduction, therefore, is that the *function of Neumann's sheath is to limit the nutrient fluid to the circumtubular space found outside of the odontoblastic tubes.*" If a consensus of opinion could establish a fact, the existence of the sheath of Neumann would now be a fact.

When the evidence in favor of the existence of a sheath of Neumann is considered, we find it substantiated by the following factors: 1. When viewed under a microscope, a thin section of dentin shows dark rings enclosing the lumena of the tubuli. 2. When suitable stains are applied, an area directly surrounding the lumena of the tubules assumes a hue deeper than that of the surrounding matrix. 3. A separate sheath-like structure can be isolated by maceration with the aid of acids or alkalies.

Comment. (1). By directly transmitted light, a more-or-less dark ring may be noticed around the lumena of the tubuli. In our experience, the thickness of this ring changes, with change in focus. By careful focusing, these rings may disappear. *Fig. 1* is a photomicrograph of a transverse section of dentin, magnified × 6000±, where no rings around the tubuli are discernible; by slightly changing the focus, dark rings may be produced. Such rings do not prove the existence of a separate structure surrounding the dentinal tubuli. In view of the capillary size of the tubules, the rings must be regarded rather as optical phenomena caused by diffraction resulting from interference of light waves between the opening of the tubuli and the observer's eye. When diffuse instead of direct light is used, this interference, although it remains, becomes less noticeable. At certain places, a crescent-shaped shadow is thrown within the outlines of the tubules. This shadow represents, however, the inner aspect of the tubuli that are obliquely cut; often partly encircles the inner aspect of the tubuli at the same sides in the sections; and looks like a circumtubular structure (*fig. 2*).

(2). Orban (20), in his Dental Histology and Embryology (1927),

does not take a definite stand as to the existence of the sheath of Neumann, but says: "It is a fact that, in stained sections of dentin, the walls of the tubules stain darker than the rest of the dentin matrix." Many other authors describe the difference in staining reactions of the layers immediately surrounding the lumena of the dentinal tubules compared with those of the rest of the dentin matrix. In a discussion (21) of a paper by Bödecker (22), I expressed the belief that the difference in staining is due to double staining. When a section of dentin is immersed in hematoxylin and eosin, or in any other staining fluid, the section is stained on both sides; and the circumtubular area imbibes the stain from within the tubule, so that, under the microscope, the total amount of staining around the tubuli is greater than that of the rest of the matrix. In order to test this point, we have produced, in celloidin, an enlarged likeness of dentin, and have subjected these perfectly homogeneous sections to different staining processes. We found each tubule in our replica to be surrounded by a circularly-stained area comparable with the staining effects attained in dentin (*figs. 3, 4* and *5*). The capillary nature of dentin structure causes stains in ordinary staining processes to remain effective within the section for a longer time than on its surface, where stains are easily washed off. We believe this simple experiment shows that the deeper staining in circumtubular areas is not due to the existence of a separate structure, nor to the presence of even a layer of different density.

(3). As to the third phase of evidence on which the existence of tubular sheaths is based, Kölliker took a stand against a previous interpretation of structures isolated from dentin by maceration. He did not consider these structures to be dentinal tubuli, and believed that concentrated acids or alkalies would certainly dissolve the tubular walls. Bödecker and Applebaum (23) presented a photomicrograph of a section of dentin treated with 50 percent nitric acid, and again interpreted the remains after maceration as sheaths of Neumann. We agree with Kölliker that the dentinal fibril, which is strongly resistant to mechanical and chemical agencies, should resist the effects of maceration. Several times we have seen dentin matrix reduced to a soft mass under the action of acids and alkalies, and have isolated the supposed dentinal tubuli. We have hesitated for many years to interpret the nature of these isolated structures, but have finally come

to the conclusion that correct focusing in diffuse light shows them to be dentinal fibrils. The fine lines which, at a certain focus, accompany these fibrils and must again be ascribed to difraction phenomena, then merge with the plasmolemma of the fibrillar cytoplasm, and single outlines are obtained. Meyer (24), in his Lehrbuch der normalen Histologie und Entwicklungsgeschichte der Zähne des Menschen (1932), gives on page 59 two excellent illustrations of these isolated fibrils, photographed in diffuse and direct light (*figs. 6* and 7). *Fig. 6* shows a single outline of the plasmolemma, whereas in *fig.* 7, difraction of the light-pencils has doubled these outlines.

Conclusions. Misleading optical phenomena and double-staining processes have hampered investigators in determining the nature of circumtubular areas in dentin. Structures resisting maceration of dentin must be interpreted as dentinal fibrils. Unless definite proof of the actuality of a sheath of Neumann is presented, its existence cannot be accepted.

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CHEMICAL ANALYSIS OF TOOTH SAMPLES COMPOSED OF ENAMEL, DENTINE, AND CEMENTUM[1]

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Introduction. Although many investigators have analyzed enamel, dentine, and cementum of teeth, separately, a smaller number have analyzed whole teeth in which enamel, dentine, and cementum occurred in unknown proportions. Results of recorded analyses on whole teeth are summarized in *table 1*, which includes indications of the materials analyzed. Most of the authors did not report results in terms of percentages of Ca (6), P (4), Mg (5), CO_2 (7), H_2O (1), organic matter (2), and inorganic matter (3), but percentages in the table are recalculated from data for tooth composition as originally expressed, to make the values comparable. Whenever the basis of the original calculations was stated, i.e., percentage of ash-weight, percentage of dry-weight, etc., that basis is noted by a suffixed letter, as indicated in the footnote under the table. The reported data, though abundant, lead to conclusions that are in some cases self-contradictory, in others radically discordant, and never completely congruent; and have been used to support inclusive and sweeping theoretical inferences. Thorough consideration of the data is out of place here, but among the sources of contradictions are these: (a) materials not described, (b) precision of methods not ascertained, (c) insufficient number of analyses, (d) insufficient precision of analyses, (e) conclusions not checked by known statistical principles. Our own results are offered, not in the belief that they remove the contradictions, but to (a) increase

[1] The data in this paper are taken from a thesis submitted by Mr. Crowell in partial fulfillment of the requirements for the degree of Master of Science, at the University of Rochester.

[2] Deceased.

[3] Rockefeller Senior Fellow in Dentistry.

available information, (b) give evidence on current problems, and (c) serve as the basis for calculation of a new tentative formula representing the inorganic part of tooth substance. Our study is part of a more inclusive research in progress. The data are not presumed to be final, and their conclusiveness does not extend beyond the indicated number of results. Since the analyses have been carried out by one of us (C.D.C.) whose work is finished, it is expedient to report the available results.

Experimental procedure. Teeth, obtained from the extraction clinic of the Strong Memorial Hospital, were immersed in tap-water until preliminary procedures could be applied. The portion of the tooth subjected to chemical analyses was that remaining after a slab about 1 mm. thick had been cut from the center of the crown, and consisted of a mixture of enamel, dentine, and cementum of unknown proportions—different, however, from proportions in which these tissues occur in the intact tooth.[4] All pulp was removed, and also any gingival tissue or decay, by scraping with a knife edge or dental spoon-shaped excavator. The specimen was rinsed first with 95 percent alcohol and then with ether. The cleaned specimens were weighed (moist weight), and transferred to a constant-temperature oven at 96°C., where they were kept for at least seven days, when they were removed, allowed to cool, and dry weight obtained.

By modification of Gabriel's method (3), organic matter was extracted to leave an inorganic residue. The specimen in a 200-cc. flask, and covered with approximately 30 cc. of a 3 percent solution of KOH in ethylene glycol, was placed upon a heater, the liquid boiled gently for approximately 90 minutes (maximum negative variation, 15 minutes), then allowed to cool and poured off; and the specimen, after rinsing with distilled water, was washed into a beaker with two portions of distilled water. In the earlier part of the research, glycerol (3, 15) was used instead of glycol, and the flask heated upon a dirt-bath at 250°C. for 90 minutes over a Bunsen flame. This

[4] The method of removing the "slab" from the "center" of the crown, as well as the purpose of its removal, are indicated in detail in "A quantitative method for studying the roentgen-ray absorption of tooth slabs," by Warren, Bishop, Hodge, and Van Huysen, *Amer. Jour. of Roentgenology and Radium Therapy, 31,* 663 (1934); and in "Quantitative roentgen-ray study of certain pathological changes in dentin," by Van Huysen, Hodge, Warren, and Bishop, *Dental Cosmos, 75,* 729 (1933). The purpose of the removal, stated quite generally, was to supply material for the studies described in the first of these papers.

TABLE 1

Results of analyses of tooth tissue (mixture: enamel, dentine, and cementum)[1]

INVESTIGATOR	MATERIAL (TOOTH)	WATER	ORGANIC	INORGANIC	P	Mg	Ca	CO_2
		percent	*percent*	*percent*	*percent*	*percent*	*percent*	*percent*
Pepys (1)	Human adult	10	20		12.8		27.2	3.6
1814	Deciduous	12	20		12.4		26.4	3.6
Lassaigne (2)	Human adult		10		12.2		27.6	4.4
1821	6-year child		28.5		12.0		27.8	5.1
Gabriel (3)	Ox	3.48A[1]			17.00A	0.91A	36.1A	4.09A
1894								
Gassmann (4)	Human incisor	8.09	22.20L		13.40	0.87	29.78	3.07
1908	deciduous	8.76	22.84L		13.32	0.78	29.59	3.03
	third molar	6.91	18.33L		13.58	0.80	31.65	3.82
	adult	8.27	21.42L		13.42	0.82	30.25	3.16
	Dog	10.97	25.99L		12.80	0.75	27.23	3.30
	Prehistoric		24.90L		12.75	0.28	29.94	2.98
	Modern		22.20L		13.40	0.87	29.78	3.06
Ulrich (5)	Human; pyorrh.:							
1925	11 sound					0.92	38.1	
	13 carious					0.72	36.3	
	Non-pyorrh.:							
	9 sound					0.89	37.2	
	11 carious					1.25	36.8	
Howe (6)	Guinea pigs:							
1926	14 scorbutic					2.881		
	controls					1.085		
Matsuda (7)	Rat: 23 days	18.8	20.3	60.9M	12.7	1.16	21.8	
1926	150 days	13.7	17.7	68.6M	13.4	1.51	24.1	
Toverud (8)	Guinea pigs:							
1923	7 controls			73.2A	19.0A	1.65A	35.3A	
	10–1 preg.			65.2A	18.9A	1.04A	37.7A	
	12–2 preg.			56.4A	18.9A	0.73A	38.7A	
1927	4 normal			78.58A		1.74A	27.6A	
	15 scorbutic			73.76A		3.98A	33.6A	
Reeves (9)	Human: 20 incisors						24.5	
1928	20 molars						27.6	
Kaushansky	Human:							
(10)	23 sound; crown						31.55	} 28.46
1930	sound; root						25.37	
	14 carious; crown						29.74	} 27.66
	carious; root						25.59	
	31 pyorrh.; crown						30.28	} 27.84
	pyorrh.; root						25.41	
Karshan (11)	Rats: rachitic				13.8±0.5		26.7±0.6D	
1930	rach., $+KH_2PO_4$				13.8±0.6		26.2±0.8D	
	rach., $+KH_2PO_4$							
	+CLO				14.0±0.1		26.5±0.5D	
Bergamini	Human; decid.							
(12)	sound; crown						26.4–29.3A	
1932	carious; crown						22.2–25.7A	
Kaushansky	Human:							
(13)	22 sound; crown			14.32	15.65D	0.50D	} 0.60	
1932	sound; root				13.00D	0.71D		
	14 carious; crown			15.18	16.21D	0.62D	} 0.67	
	carious; root				14.14D	0.71D		
	21 pyorrh.; crown			14.62	16.03	0.68D	} 0.96	
	pyorrh.; root				13.21D	1.25D		
Quintarelli	Human: first molar						27.05	
(14)	mated 2nd molar						27.15	
1932								

[1] *A* indicates percentage on basis of ash weight; *D*, dry weight; *M*, moist weight; *L*, loss on incineration.

procedure was difficult; temperature fluctuations necessitated frequent readjustments of the flame, and overheating was common. Overheated specimens were darkened in the dentinal portion, the color in some cases

not being removed by subsequent washings. These difficulties led to search for a liquid that could be used at its boiling point. Glycol was found to be satisfactory. The 500-watt type RH-heaters (Precision Scientific Co.) can be very easily adjusted to produce steady boiling at 205°C. Micro-Nessler determinations for N were performed by Clara Husted Present upon extracted residues from four teeth selected at random. Negative results in each case showed complete absence of protein, indicating that glycol solution of KOH satisfactorily removed all organic matter.

In extraction of organic matter with KOH, the inorganic residue becomes permeated by the base, which is subsequently extracted with boiling distilled water. The specimens were covered with about 200 cc. of distilled water and placed upon a hot plate. After approximately 30 minutes of boiling, the supernatant solution was decanted, and the specimens again covered with the same volume of distilled water and placed upon the hot-plate. The boiling and decantation were repeated three times, the supernatant liquid after the fourth extraction being colorless to phenolphthalein. The inorganic residues were placed in a constant-temperature oven, and kept there over night. On the following day they were removed, allowed to cool, and the inorganic weight determined. Our attention was privately directed by Prof. S. A. Pond to the possibility that calcium phosphates of the teeth are hydrolyzed during the washing with water following extraction. Hydrolysis would tend to produce soluble phosphates, which would be lost with the washings, and erroneous values thereby obtained for percentages of Ca, P, and Mg, as well as for the Ca:P ratio. He also pointed out that such decomposition could be avoided by washing with isopropyl alcohol, which is a good solvent for KOH but in which calcium phosphates are highly insoluble. In an effort to determine any difference, the inorganic residues of six teeth were extracted with water, and the inorganic residues of four teeth were extracted with isopropyl alcohol, on a hot-plate using reflux condensation. The teeth in both series were then analyzed according to the regular routine, with results given in *table 2*, which show that there was no significant difference for the criteria used. Therefore, it is concluded that, if phosphates of the inorganic residue of a tooth are not hydrolyzed by isopropyl-alcohol extraction, they are probably not hydrolyzed appreciably by water-extraction, under the conditions of our work.

The specimens were next pulverized in a porcelain mortar. The powder was brushed into a tared weighing bottle, and its weight determined; then transferred, through a powder funnel, to a volumetric flask, excess of standard HCl (approximately 0.2 *N*) added from a pipette of known volume, and any particles of powder adhering to funnel or neck washed down. Acid adhering

to the funnel or neck was washed in with distilled water. When all material was dissolved, the solution was made up to volume with water. In many cases a very small, insoluble residue (possibly calcium fluoride) was observed, even in the presence of an ample excess of acid; in no case did it amount to more than about 2 cmm. P was determined colorimetrically by the method of Fiske and Subbarow (16); Mg, by the titration method of Tisdall and Kramer (17); Ca, by the titration method of Clark and Collip (18). The aliquots were chosen to contain approximately the following amounts of proper constituent: P, 0.4 mg.; Mg, 0.0 to 1.5 mgs.; Ca, 2.0 to 3.6 mgs. The precisions of the methods, expressed as possible deviations between results on duplicate aliquots, are 3, 4, and 2 percent respectively, of the

TABLE 2

Data on inorganic residues washed with different solvents

TOOTH NO.	SOLVENT	P: PERCENT OF INORG. WT.	Mg: PERCENT OF INORG. WT.	Ca: PERCENT OF INORG. WT.	BASIC EXCESS	Ca:P
101	Isopropyl alc.	15.9	0.206	37.6	13.9	2.37
103	Isopropyl alc.	15.3	0.097	37.5	13.5	2.45
104	Isopropyl alc.	15.7	0.163	37.5	19.8	2.38
105	Isopropyl alc.	16.0	0.237	39.9	13.8	2.49
111	Water	16.0	0.017	37.9	13.7	2.37
112	Water	16.2	0.231	38.1	16.3	2.35
114	Water	15.7	0.105	42.0	13.5	2.67
115	Water	16.4	0.0865	39.7	13.5	2.42
117	Water	16.7	0.295	39.6	12.4	2.38
118	Water	16.8	0.339	39.4	13.7	2.67

actual values for P, Mg, and Ca. "Basic excess" was determined in aliquots of solution containing inorganic sample, delivered into flasks and aqueous methyl-orange added to each. Standard NaOH (approximately 0.1 *N*) was then run in, with constant rotation until the solution acquired a salmon color, when the flask was placed upon a hot-plate, contents boiled for a few seconds, then allowed to cool, and titration finished at room temperature (end-point, first disappearance of distinct salmon color). It has been shown experimentally that further addition of base causes precipitation of phosphates. Consequently the NaOH used was regarded as equivalent to the excess of HCl in the aliquot over that needed to dissolve the inorganic residue. The "basic excess" in the inorganic residue is calculated as liters of *N* HCl required to dissolve 1000 gms. of inorganic residue (15).

Description of data. The data determined directly were moist weight; dry weight; inorganic weight; percentages of P, Mg, and Ca on the basis of inorganic weight; and "basic excess" in inorganic residue, according to definition. All other percentage data, including water, inorganic matter on basis of moist and dry weights, extracted matter on basis of moist and dry weights, and P, Mg, and Ca on basis of moist and dry weights, were obtained indirectly by calculations. The total number of teeth was 139. In addition to analytical data, a number of facts about each analyzed tooth are also recorded, among which the following are noted in *table 3*: age, sex, kind and condition of tooth. Percentages of water, inorganic residue, P, Mg, and Ca, and "basic excess," are given in *table 4*. Percentage of water is of moist weight; percentage of inorganic residue is of dry weight; percentages of P, Mg, and Ca are of the inorganic residue.

Since the analyses for P, Mg, and Ca were made on a sample of inorganic residue, the results are representative of the inorganic part of the whole tooth irrespective of the proportions of enamel, dentine and cementum. To establish this contention it is only necessary to recall that each of the three tissues involved may be considered as being made up of water, organic matter, and inorganic matter. All water and organic matter having been removed, only inorganic matter —"residue"—remains. All data from a variety of experimental studies, i.e., chemical, roentgenographical, crystallographical, etc., indicate that the inorganic parts of tooth tissues, whether enamel, dentine, or cementum are one and the same substance. Thus the analytic results for Ca, P, and Mg are significant for the intact tooth.[5]

Not all analyzed teeth are included in the record. We discarded values whose divergence from averages was greater than three times standard deviations. Averages and standard deviations in *table 2* were calculated for each component, using total number of values indicated. *Tables 4* and *5* show that values for percentages of water and inorganic residue vary through widely separated limits: of water, from 4.0 to 14.3—average, 9.17; of inorganic residue, from 67.6 to

[5] For a brief review of the crystallographical and roentgenographical studies see "Roentgen-ray diffraction studies of enamel and dentine," by Bale, Hodge, and Warren, *Amer. Jour. of Roentgenology and Radium Therapy* (in press). For chemical studies, the bibliography of the present paper will serve as an introduction.

TABLE 3

Data on age and sex of patient and kind of tooth, for teeth whose analyses are given in table 5

TOOTH NO.	AGE	SEX	KIND[1]	CONDITION[2]	TOOTH NO.	AGE	SEX	KIND[1]	CONDITION[2]
55	25	M	L R 4	S	99	55	M	U R 5	S
56	25	M	L R 5	S	100	58	M	U R 5	S
57	19	F	L L 6	S	101	58	M	U R 8	S
58	39	F	U L 3	S	103	37	M	L R 7	S
59	30	M	U R 1	S	104	26	F	L L 5	S
61	30	M	U R 3	C	105	27	M	U R 5	S
64	49	M	L L 1	S	109	32	F	U R 1	S
65	26	F	L R 8	S	110	32	F	L R 4	S
66	48	M	U R 9	S	111	38	F	L R 3	S
67	58	M	L L 3	S	112	38	F	L R 2	S
68	58	M	L L 4	S	114	42	M	U L 7	C
71	36	M	L L 3	S	117	..	..	U L 5	S
72	36	M	L L 2	S	118	..	..	U L 4	S
75	21	F	L R 6	S	119	..	..	U L 3	S
76	21	F	L R 7	S	120	..	..	U L 1	S
77	21	F	L R 4	S	121	..	..	U L 2	C
78	21	F	L L 8	S	122	..	..	U R 4	C
79	58	M	U R 7	S	123	..	..	U R 6	C
80	50	M	U R 1	S	125	..	..	L L 3	S
81	50	M	L R 3	S	126	..	..	L L 2	S
82	50	M	U R 3	S	127	..	..	L L 1	S
83	50	M	U L 3	S	128	..	..	L R 1	S
84	50	M	U R 2	S	129	..	..	L R 2	S
85	50	M	L R 7	S	130	..	..	L R 3	S
86	50	M	U L 2	S	132	26	F	U L 6	C
87	50	M	U R 4	S	133	26	F	U L 7	C
88	50	M	U L 1	S	134	26	F	U L 8	C
89	50	M	L R 1	S	135	26	F	U R 5	S
90	50	M	U L 4	C	136	26	F	L R 5	S
91	50	M	L R 4	S	137	26	F	U L 5	S
92	45	F	U L 5	S	138	26	F	U R 4	S
93	45	F	U L 6	S	139	26	F	U R 3	S
94	45	F	U L 7	S	140	26	F	U R 2	S
95	45	F	L R 1	S	141	26	F	U R 1	S
96	45	F	L L 1	S	142	26	F	U L 1	S
97	45	F	L L 2	C	143	26	F	U L 2	S
98	45	F	L L 3	C	144	26	F	U L 3	S

[1] Kind of tooth: U = upper; L = lower; R = right; L = left; 1 = central; 2 = lateral; 3 = cuspid; 4, 5 = bicuspids; 6, 7, 8 = molars.

[2] Condition: S = sound; C = carious.

TABLE 4

TOOTH NO.	WATER: PERCENT OF MOIST WEIGHT	INORG. RES.: PERCENT OF DRY WT.	P: PERCENT OF INORG. WT.	Mg: PERCENT OF INORG. WT.	Ca: PERCENT OF INORG. WT.	BASIC EXCESS[1]
55	11.1	85.2	16.3	0.502	42.2	5.91*
56	11.2	84.7	16.0	0.557	39.4	13.7
57	14.9*	77.7	15.6	0.936	34.1	13.9
58	12.2	83.0	15.4	0.131	36.9	7.33
59	9.9	68.6	16.0	0.519	36.1	13.7
61	9.8	36.7*	15.7	0.0218	37.9	14.8
64	8.9	28.3*	15.7	0.884	..	..
65	16.1*	80.8	15.9	0.117	57.6*	9.38
66	8.7	65.9*	16.3	0.877	36.9	12.1
67	8.9	91.5	16.0	0.236	29.5	13.4
68	9.5	..	16.5	1.07	38.0	7.08
71	17.0*	88.2	16.2	0.825	32.9	10.3
72	10.0	..	15.2	0.959	..	12.6
75	9.4	83.6	17.0	0.210	9.55*	13.5
76	10.2	88.4	16.4	0.125	37.7	..
77	8.6	88.8	16.5	0.787	37.3	7.25
78	5.6	77.6	16.5	1.29	37.1	9.50
79	8.3	..	..	..	..	..
80	4.4	81.8	16.0	0.0245	36.3	13.1
81	3.4*	81.5	16.2	0.231	36.1	13.1
82	7.1	..	16.7	0.719	37.5	..
83	7.4	..	..	..	..	..
84	5.2	82.0	17.0	0.0691	36.7	13.5
85	5.8	87.5	16.9	0.997	36.7	..
86	3.2*	81.6	16.6	0.0878	38.9	13.5
87	4.4	83.5	16.9	0.533	38.3	13.3
88	4.7	82.1	16.2	0.451	37.6	13.9
89	7.7	..	18.2	0.650	38.3	13.6
90	6.4	82.5	16.3	0.526	38.7	13.3
91	4.4	83.7	16.3	0.415	37.4	16.5
92	5.0	88.7	..	0.0418	57.4*	13.5
93	5.7	89.1	16.9	0.495	39.7	5.08*
94	4.8	86.0	17.7	0.0390	37.1	7.37
95	7.7	84.9	15.5	0.335	34.9	13.9
96	4.0	..	15.5	0.834	35.9	13.4
97	4.7	77.5	16.0	0.703	36.5	17.3
98	8.5	85.5	16.3	0.863	40.1	13.3
99	7.5	83.5	17.4	0.825	38.3	21.1*
100	5.6	80.3	14.8	0.579	36.7	13.1
101	7.9	91.5	15.9	0.206	37.6	..
103	11.4	95.1	15.3	0.097	37.5	13.5
104	9.8	95.4	..	0.163	37.5	..
105	10.7	..	16.0	0.237	39.9	13.8
109	..	..	..	0.304	40.4	9.43
110	..	..	16.4	0.171	42.4	17.1
111	..	86.9	16.0	0.017	37.9	13.7
112	..	87.9	16.2	0.231	38.1	..
114	..	87.3	15.7	0.105	42.0	13.5
115	9.1	87.5	16.4	0.0865	39.7	..
117	9.4	42.8*	16.7	0.295	39.6	12.4
118	9.9	..	16.8	0.339	..	13.7
119	10.9	87.3	17.0	0.229	39.3	13.0
120	9.2	85.0	17.3	0.181	40.1	8.62
121	12.3	81.8	17.1	..	..	..
122	10.1	83.2	12.1*	0.188	39.5	9.52
123	9.2	78.0	15.5	0.205	..	15.5
125	10.0	84.8	..	..	..	..
126	8.7	78.9	17.1	..	..	13.7
127	9.8	79.0	16.4	0.682	..	..
128	9.7	..	..	..	..	..
129	10.0	85.6	16.4	0.199	40.1	13.9
130	10.5	88.3	16.2	0.179	40.3	13.3
132	9.7	87.2	16.7	0.556	41.7	16.5
133	9.4	80.8	16.5	0.794	41.7	23.0*
134	10.1	89.3	11.3*	0.129	..	15.0
135	11.2	84.3	16.7	..	42.4	..
136	10.1	87.0	..	0.591	38.3	..
137	9.0	..	..	..	..	..
138	8.1	87.3	17.1	0.651	..	..
139	7.7	91.0	..	0.648	38.0	..
140	9.7	87.8	16.5	0.951	36.8	..
141	9.2	..	..	..	..	..
142	9.1	..	..	..	..	..
143	10.5	85.8	17.1	1.05	37.1	..
144	10.1	..	..	..	..	..

[1] Liters of *N* HCl used to dissolve 1000 gms. of inorganic residue.
* Values discarded, on basis of statistical rules, before computing averages.

95.7—average, 81.6. The range for percentage of P, 13.4 to 19.2 about an average of 16.3, is much narrower; of Ca or Mg, it was so great that certain values had to be discarded to assure a true average; that is to say, certain values are so far removed from the average that there is only a very small probability that those values are true, consequently they were discarded. The number of percentage values discarded, on the bases of standard deviations, are: water, 8; inorganic residue, 6; P, 9; Mg, 21; Ca, 23; "basic excess," 18. That these values are far from the average may be due to accident, experimental error, mistake, real variation in specimens, or other causes. It is common statistical procedure to discard values according to the rules mentioned above. After discarding atypical values, the ranges about average

TABLE 5

Condensed account of complete analytical results

	WATER: PERCENT OF MOIST WT.	INORG. RES.: PERCENT OF DRY WT.	P: PERCENT OF INORG. WT.	Mg: PERCENT OF INORG. WT.	Ca: PERCENT OF INORG. WT.	BASIC EXCESS
General average	9.2	81.6	16.3	1.39	34.5	11.5
Standard deviation	1.72	4.68	0.86	1.70	23.6	2.48
Total no. of values	107	86	104	104	90	70
No of atypical values	8	6	9	21	23	18
Average after discarding atypical values				0.32	37.5	12.7

percentage values for Mg and Ca are as follows: Mg, 0.0 to 1.8—average, 0.32; Ca, 27.0 to 47.0—average, 37.5. The limits for "basic excess" are 6.5 and 17.5—average, 12.7.

To obtain a concise picture of the data, let it be said that on the basis of tabulated analyses, percentage values are: water, 9.2 ±1.7; inorganic residue, 81.6 ±4.7; P, 16.3 ±0.9; Mg, 0.32 ±0.25; Ca, 37.5 ±9.8; "basic excess," 12.7 ±2.5. (The significance of average ± standard deviations is this: about 70 percent of the values taken to form an average lie within the limits indicated.) The most outstanding fact in *table 5* is the usual constancy of percentage values for P; intermediate in variability are those for Ca; the values for Mg are the most variable. High values for Mg are frequently associated with low values for Ca and "basic excess." By comparison with *table 1.*

it may be seen that values in *table 5* are not much different from those reported by other investigators, excepting the average for Mg, which is lower than most values hitherto given.

Description of data according to various groupings, i.e., sound vs. carious, male vs. female, lustral age groups. The data for teeth 54 to 150, excepting 115 to 131, were tabulated (*table 6*) to favor comparison of

TABLE 6

Data tabulated according to various groupings

GROUPS	WATER: PERCENT OF MOIST WEIGHT	INORG. RES.: PERCENT OF DRY WT.	P: PERCENT OF INORG. WT.	Mg: PERCENT OF INORG. WT.	Ca: PERCENT OF INORG. WT.
Gen. average, *table 5*	9.2 ± 1.7	81.6 ± 4.7	16.3 ± 0.97	0.32 ± 0.25	37.5 ± 9.8
Sound	8.1	86.1	16.4	0.490	38.1
	(45)	(35)	(36)	(39)	(36)
Carious	9.1	73.9	16.0	0.480	36.5
	(15)	(9)	(14)	(15)	(12)
Male	7.9	84.6	16.2	0.531	37.9
	(26)	(20)	(28)	(35)	(32)
Female	8.9	85.7	16.3	0.474	38.4
	(31)	(26)	(23)	(28)	(24)
20–25 years	10.7	85.5	16.5	0.579	38.5
	(7)	(7)	(7)	(6)	(6)
25–30 years	9.8	85.3	15.9	0.494	40.4
	(22)	(11)	(11)	(13)	(12)
35–40 years	12.7	86.4	15.7	0.377	36.7
	(4)	(3)	(6)	(6)	(5)
40–45 years	5.8	85.6	16.2	0.427	38.0
	(7)	(7)	(7)	(8)	(7)
45–50 years	6.1	81.2	16.7	0.497	37.4
	(14)	(10)	(13)	(13)	(12)
55–60 years	8.0	87.7	15.8	0.523	35.4
	(5)	(3)	(4)	(4)	(4)

average values for the following groups: sound and carious teeth, male and female, lustral age-groups. Figures in parentheses indicate number of values used to compute each average. The "general average" for each constituent is taken from *table 5*.

Variations in percentage composition of sound versus carious teeth. From *table 6* it may be seen that *sound* teeth have high values for inor-

ganic residue—average for 35 teeth, 86.1, as compared with the general average of 81.6. *Carious* teeth show a low value for inorganic residue —average for 9 teeth, 73.9; and have normal average values for P, Mg, and Ca (basis of inorganic weight). The reports to date regarding differences between carious and non-carious teeth may be briefly scanned. *Carious* tooth-tissue contains *less* Ca than sound tooth-tissue (19, 12, 10, 5); *less* organic matter (19); *less* P (13, 20); *less* CO_2, and *more* water (20); carious tissue has also been reported as containing *more* Ca and organic matter (20), and *more* Mg (13, 5, 6). No differences were found between carious and sound tooth-tissues as to water, ash, and volatile matter (21), as to Ca (22), and as to Mg (20). Many apparent discrepancies were due to use of samples that included portions infected by caries. If our figures are recalculated on the basis of moist weight of tooth sample, percentage differences for Ca, Mg, and P—for sound and carious teeth—are similar to the above-mentioned difference in inorganic content. However, since these values are reported as percentages of inorganic residue, they represent also samples from which all carious material had been removed before analysis—hence no difference between sound and carious dentine. Our data may thus be said to agree with previous results.

Variations in percentage composition of male versus female teeth. It may be seen also from *table 6* that average values for each constituent are about the same, whether the tooth came from a male or a female patient. Von Bibra (23) reported small differences in composition of enamel and dentine of adult male and female teeth. However, Black (21) found no difference between sexes in ash content, volatile matter, and water content of dentine for the same age-groups, and Fish and Ferguson (22) observed no sex difference in Ca content of dentine from different sexes in the same age groups. The data in *table 6* confirm and agree with the results of previous investigators.

Variations in percentage composition of teeth with age increase. *Table 6* indicates that there is little difference between average values when computed for lustral age-groups from 20 to 60 years. This agrees with Lassaigne's results (2), which show slight chemical difference between a permanent tooth of a 6-year child and one of an adult. However, data have been reported to the effect that, with increasing age, there were increases in ash (21, 7), P (24, 7), Ca (24,

7, 25, 22) and Mg (7). Other data show that, with increasing age, there were decreases in volatile or organic matter (21, 24, 7), water (21, 24, 7), Mg (24, 25), ash (25), and chloride (25). Owing to insufficient teeth in each age-group, and probably also to lack of precision in methods, the data in *table 6* cannot be said to controvert any conclusions stated by other investigators. There is some histological evidence that teeth "mature" (26) with age, but it is open to question whether this maturation is evidence of change in chemical composition of the inorganic portion.

Variations in percentage composition of teeth from the same individual. *Table 7* was prepared to show data for teeth from four individuals.

TABLE 7

Data on teeth grouped according to individuals

PATIENT	WATER: PERCENT OF MOIST WT.		INORG. RES.: PERCENT OF DRY WT.		P: PERCENT OF INORG. WT.		Mg: PERCENT OF INORG. WT.		Ca: PERCENT OF INORG. WT.	
	Av.	Range	Av.	Range	Av.	Range	Av.	Range	Av.	Range
D. F.	8.0 (5)	3.9	87.8 (3)	11.2	15.9 (4)	1.7	0.523 (4)	0.864	35.4 (4)	8.5
H. S.	5.7 (10)	3.3	82.9 (9)	6.0	16.7 (11)	2.0	0.421 (11)	0.766	37.5 (11)	2.8
G. U.	10.0 (13)	3.6	83.2 (10)	10.3	16.7 (10)	1.8	0.277 (9)	0.503	39.8 (6)	1.0
M. B.	9.3 (15)	3.5	86.7 (9)	6.7	16.8 (6)	0.6	0.671 (8)	0.921	39.4 (7)	5.9
Gen. av.	9.2± 1.7		81.6± 4.7		16.3± 0.97		0.323±0.25		37.5± 9.8	

Averages were calculated for each component, and arranged according to names of patients. Values in parentheses indicate number of teeth upon which averages are based. The "general average" for each constituent is taken from *table 5*. A brief summary for each individual follows: *Patient D. F.*—Ca is fairly constant, having less variation than the standard deviation of the general average; all other constituents vary between limits of over twice the standard deviation. *Patient H. S.*—Ca has a high degree of constancy; all other constituents vary widely. *Patient G. U.*—Mg is fairly constant; Ca is remarkably constant; all other constituents vary widely. *Patient M. B.*—P is unusually constant; all other constituents vary widely.

These results do not indicate that the teeth in the individual tend to be constant or characteristic in composition. In fact, teeth from the same mouth show greater variation in composition than the averages for many teeth from different individuals. Comparing the same tooth constituent for different individuals, *D. F.*, *G. U.*, and *M. B.* have normal values for water; *H. S.* has low values. *D. F.* and *M. B.* show high values for inorganic matter; *H. S.* and *G. U.* have normal values. In the case of P, the averages for all individuals are normal, that for *D. F.* being smaller than the others. Mg for *D. F.* and *M. B.* is a little high, as compared with the general average; for *H. S.* and *G. U.* it is normal. All averages for Ca are normal. In spite of the differences between teeth in the same mouth, shown above, these variations may evidently lie about averages which differ from patient to patient. For example, water for 10 teeth of *H. S.* varies over a range of 3.3 percent, but has an average value of 5.7—very much less than the general average of 9.2; while water for 13 teeth of *G. U.* varies over a closely comparable range of 3.6 percent, but has an average value of 10.0—slightly greater than the general average, but probably normal. Inasmuch as no other investigator has pointed out this type of variation (and because results from only four patients are given), further study is necessary before the conclusion can be accepted as valid. However, this type of difference may be a contributing factor in the susceptibility of all teeth of certain individuals to caries; while widely varying compositions of teeth in the same mouth, as above demonstrated, may help to explain why in other individuals only one or two teeth are susceptible.

Variations in percentage composition of teeth as a function of tooth type. A chart was made for each of three individuals (*H. S.*, *G. U.*, and *M. B.*), plotting the values for P, water, inorganic matter, Mg, and Ca against type of tooth. In no case could a relation be seen between type of tooth and constituent. However, for no individual were the analyzed teeth numerous enough to justify the conclusion that there is no such relation. Charts were also made from data on a large number of teeth from different individuals, plotting type of tooth against the various constituents. In no case could any relation be seen between type of tooth and constituent, although too few teeth were studied to permit the conclusion that there is no relation between

tooth type and composition. In general these results verify previously announced statements by other investigators. Toverud (8) showed that, for guinea-pig teeth, molars and incisors have approximately the same ash yield, but that molars are richer in Ca, and poorer in P and Mg, than incisors. Matsuda (7) found that, for rat jaws, teeth in the upper jaw have higher contents of organic matter, water, and Mg, and lower yield of ash and content of Ca, than teeth in the lower jaw; while content of P is about the same for upper and lower. Reeves (9), analyzing 20 human incisors and 20 molars, found Ca content of molars greater than that of incisors. Quintarelli (14) could show no regular difference in Ca content in first and second permanent molars on the same side of the same jaw, in 25 such pairs studied. Fish and Ferguson (22) reported Ca contents in 4 incisors, 9 canines, 35 premolars, and 16 molars, without establishing any regular differences; there was also no regular difference between the two teeth of the same sort from opposite sides of the same jaws, in four such pairs. Montelius (27) presented data in which there is no regular difference in contents of volatile matter, Ca, and P in enamel from human molars and incisors.

Calculation of a tentative formula for the inorganic residue. The significance of data in *table 4* for "basic excess" is not at once apparent. Broadly speaking, "basic excess" suggests certain conclusions regarding molecular constitution of the inorganic residue of tooth tissue. Many and diversely determined facts, which must be considered and organized to give a composite picture of this molecular structure, form a background too complicated to be considered at this time. It will be sufficient to show how values for "basic excess" may be employed in the calculation of a *tentative* empirical formula. It has been known, for some time, that the inorganic portion is not tricalcium phosphate nor a mixture of tricalcium phosphate and calcium carbonate, although Ca, phosphates, and carbonates are the principal constituents. X-ray spectra show that the arrangement of Ca, P, C, and O atoms is such as to preclude the presence of either of the first named substances as the principal component. Instead, the arrangement is that of a crystal of great complexity, whose exact dimensions and space relations are so intricate that they are of interest only to crystallographers. However, since many theories regarding dental

caries or dental health involve the nature of the principal constituent of the inorganic portion, it is important to know that the principal crystalline constituent seems to belong to the group of minerals called the apatite series (28), whose composition has a complex proportion of Ca, phosphate, and other atoms or atomic groups sometimes including carbonates. The description so far given, while true, is unsatisfactory and vague; consequently, to simplify and clarify the mass of information, the "basic excess" is used below to calculate a tentative formula similar to one of the formulae used in describing certain apatite minerals. By "basic excess" is meant presence of more basic than acidic material in the inorganic residue; the basic components being Ca and Mg, the acidic radical being phosphate. Carbonate is also present as an acidic radical, but was not determined. However, a fair approximate value for the CO_2-content is 4 percent (see *table 1*). Expressing the analyzed components, i.e., Ca, P, and Mg, as phosphates of Ca and Mg, the inorganic residue is roughly 80.0 percent of $Ca_3(PO_4)_2$. The 4 percent of CO_2 would require the presence of 9.1 percent of $CaCO_3$. Assuming simply that the "basic excess" is $Ca(OH)_2$, the excess of Ca would require about 5.4 percent of $Ca(OH)_2$. These compounds and proportions may be written in conventional fashion, as follows:

$$80.0\%\ Ca_3(PO_4)_2 \cdot 9.1\%\ CaCO_3 \cdot 5.4\%\ Ca(OH)_2$$

This formula may be simplified by finding the molecular proportions of each compound, with the following result:

$$3.0\ Ca_3(PO_4)_2 \cdot 1.0\ CaCO_3 \cdot 0.9\ Ca(OH)_2$$

This formula, closely approximating that ascribed to francolite, a member of the apatite series, is usually written as follows:

$$3\ Ca_3(PO_4)_2 \cdot CaCO_3 \cdot Ca(OH)_2$$

(The hydration of the crystal is not considered.) The closeness with which this formula fits the experimental data may be seen from the following comparison, which also includes a statement of some of the other formulae which have been proposed (*table 8*).

The percentage of Ca for our formula is somewhat higher than the

"general average" in *table 5*; the values for P, CO_2 + OH, and Ca:P ratio are in excellent agreement. If the inorganic residue were a compound of the formula offered, 13.3 liters of *N* HCl would be required to dissolve 1000 gms.; a value in fair agreement with those experimentally determined. Some of the various formulae heretofore offered may be briefly compared among themselves as well as with our data. Formula "A" (*table 8*) neglects CO_2-content, and does not closely correspond with our percentage values for Ca, P, or Ca:P ratio. Formula "B," based in part on x-ray study, is a better description than formula "A," but neglects the CO_2-content and has a higher P value than our data. Our formula fits our Ca:P ratio better than either "A" or "B." Formula "C" is very much like "B," with the

TABLE 8

Comparison of formulae and data

FORMULA	Ca	P	CO_2	OH	$\frac{CO_2+}{OH}$	Ca:P
	per-cent	*per-cent*	*per-cent*	*per-cent*	*per-cent*	
3 $Ca_3(PO_4)_2 \cdot CaCO_3 \cdot Ca(OH)_2$	39.2	16.6	3.6	2.8	6.4	2.36
Gen. ave.: data from *table 5*	37.5	16.3	..	..	7.2	2.31
Formula "A"—6 $Ca_3(PO_4)_2 \cdot Ca(OH)_2$: (15) and others	39.3	19.2	..	1.75	..	2.04
Formula "B"—3 $Ca_3(PO_4)_2 \cdot Ca(OH)_2$: (29) and others	39.8	18.5	..	3.39	..	2.15
Formula "C"—3 $Ca_3(PO_4)_2 \cdot CaCO_3$: (30) and others.	38.8	18.1	4.28	..	..	2.13

substitution of a carbonate for a hydroxyl radical. Calculated percentages of Ca and P for Formula "C," as for Formula "B," are higher than the analytical data; and while the calculated percentage of CO_2 is in substantial agreement with the experimental data, the hydroxyl content is neglected. The Ca:P ratio for Formula "C," closely comparable to "B," is lower than the average found experimentally. Therefore, because the formula offered here accounts for carbonate, phosphate and hydroxyl radicals, and seems to "fit" the data better than previous formulae, it is offered ***not as the formula for tooth substance***, but as a tentative empirical description of the inorganic residue.

Summary. (1) Percentage values for water, inorganic residue, P,

Mg, and Ca, as well as "basic excess," were determined for 139 tooth remnants, a slab 1 mm. thick having first been removed from the center of the crown.

(2) Age and sex of patients, as well as kind and condition of teeth, were recorded.

(3) The percentage values were (*table 5*): water, 9.2 ± 1.7; inorganic residue, 81.6 ± 4.7; P, 16.3 ± 0.9; Mg, 0.32 ± 0.25; Ca, 37.5 ± 9.8; "basic excess," 12.7 ± 2.5.

(4) The percentage of P, in inorganic residue, was least variable; of Ca, intermediate; of Mg, most variable.

(5) *Sound* teeth had a higher inorganic residue (percent of dry weight) than the average; *carious* teeth, a lower inorganic residue. Carious teeth (basis of inorganic residue) had normal average percentage values for P, Mg and Ca.

(6) No difference was shown between average composition of teeth from male and female patients.

(7) Little difference was shown between average composition of teeth from different age-groups.

(8) Variations in composition of teeth from the same mouth were as wide as for teeth from different mouths.

(9) Variations in composition of teeth from the same mouth may lie about an abnormal average; i.e., all teeth from one patient may have a low percentage of water.

(10) No relation was shown between tooth-type (morphologically) and proportion of any constituent.

(11) A *tentative* empirical formula, 3 $Ca_3(PO_4)_2 \cdot CaCO_3 \cdot Ca(OH)_2$, was proposed for the inorganic residue.

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PRESENCE OF IRON IN ENAMEL KERATIN[1]

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In a paper on "a biochemical study of the protein in dental enamel," in which it was stated that this protein is wholly or chiefly keratin, I reported some findings indicating that enamel keratin contains iron.[2] Although I endeavored to prevent adventitious introduction of iron, and the findings appeared to indicate that iron is contained in the enamel-keratin molecule, it seemed desirable, because of possibilities of unrecognized contamination, to withhold publication of details until further work could be completed. My published comment on this situation was concluded as follows (p. 206): "The details of a further study of this possibility [that enamel keratin contains iron] will be published in a succeeding paper." Unfortunately, it has been impossible for me to extend this work; but Dr. Gies, continuing his interest in the problem, initiated the study described in the next paper in this issue. At his suggestion, details that were withheld in 1930 are published below from the laboratory record.

Enamel-keratin particles, when treated with sodium sulphide in wet preparations under the microscope, immediately developed a green color. This coloration was likewise given by enamel residue from pepsin and trypsin digestions, but was not shown by dentin from a preparation containing no enamel, nor by neurokeratin or nail filings. This observation suggested the presence of iron in the enamel-residue particles, since iron sulphide in a dispersed or dilute state is green; and tests on particles with potassium thiocyanate acidified with hydrochloric acid resulted in development of the red color of iron thiocyanate, confirming this view. The experiments reported here were an effort to determine whether iron existed in the particles

[1] A part of the author's research during his incumbency of the William J. Gies Fellowship in Biological Chemistry at Columbia University: 1928–30.

[2] Rosebury: *J. Den. Res.*, 1930, *10*, 205.

intrinsically or was contributed by an external agency. Since iron instruments had been used in the preparation of the enamel, and iron was found in dust of the air and as an impurity in virtually every reagent used, elimination of all possible sources of contamination was difficult. The tests undertaken for this purpose are described serially below.

(a) *Iron in sodium sulphide solution.* Two "pure" preparations of sodium sulphide (Mallinckrodt's, labelled "iron-free;" Baker's, labelled "containing 0.005 percent of iron") both gave positive thiocyanate reactions when a solution of each was evaporated nearly to dryness and acidified. Since this iron occurred presumably as sulphide, a solution of iron sulphide was prepared by saturating dilute $FeCl_3$ solution with H_2S and filtering. The resulting green solution, which responded strongly to iron tests, failed to produce any green coloration in enamel-residue particles either alone or in presence of alkali. Thus it appeared that traces of iron in the Na_2S solution were not responsible for the coloration in the enamel-keratin particles.

(b) *Effect of contact with iron during preparation of enamel.* A specimen of enamel was prepared and put up in such a manner that contact with iron instruments did not occur at any time. The method of desiccation and fracture was employed (dentin contamination being inconsequent), but the enamel surface was protected from the pliers by two layers of washed rubber-dam and a thickness of clean cloth. The flakes were then stoned on all surfaces with carborundum, washed with 5 percent HCl, and dried on a clean watch-glass. Use of iron instruments was avoided in all incidental procedures. This specimen was demineralized in the usual way, and thereafter gave the characteristic iron reactions with Na_2S and KCNS. The iron was thus apparently not contributed by manipulation up to the point of demineralization.

(c) *Effect of other factors in manipulation.* In an effort to eliminate all sources of possible iron contamination, a special assembly was set up as follows: Glacial acetic-acid was distilled in an all-glass system and collected into a bottle which, after being used for several months to contain HNO_3, was thoroughly washed with distilled water. The distillate, after standing in this bottle for seven days, showed no trace of iron. Each piece of apparatus employed was thoroughly

washed with HNO_3, then with distilled water. Stoppers were first treated with boiling 5 percent NaOH, then with boiling 10 percent HCl, then washed with distilled water. All apparatus was finally washed thoroughly with some of the iron-free acetic acid, and then tested by standing in or with the acid for four hours, and evaporating and testing the acid used for iron. Negative results were obtained in each case. Enamel was prepared, without contact with iron, by the desiccation method mentioned above. In addition, the fragments were finally washed with some of the iron-free acetic acid, then with distilled water. All incidental operations were performed with iron-free apparatus, and without the use of filter paper (which contained traces of iron). The acetic acid was diluted for use to about 2 percent with iron-free distilled water, and the stock dilute acid kept in an iron-free bottle. This bottle and that containing glacial acetic-acid, and the cylinder of the demineralizing assembly, were kept covered with inverted beakers for protection from dust. The enamel residue obtained by this means also responded clearly to Na_2S solution, the particles taking the characteristic green color; and to KCNS and HCl, showing the typical red. These results indicate that iron is an intrinsic constituent of enamel, and not there merely as a result of contamination.

It was further observed that, although particles of enamel-residue may be charged with additional iron by contact with ferric ions, such additional iron is easily removed, whereas the iron originally present is not. Enamel, prepared by desiccation in the ordinary way, was put up in two assemblies for demineralization: one, in the usual manner; the other, having in the acetic acid enough $FeCl_3$ to color the solution. The colored acetic-acid solution was replaced after 48 hours with 0.5 percent HCl to dissolve the precipitate of ferric phosphate which had formed. Particles of enamel protein demineralized in the usual way, when subjected to the action of 10 percent HCl for ten minutes and washed in water, yielded positive tests with Na_2S and KCNS similar to those given by untreated residue. On the other hand, the demineralized residue from the system containing excess of iron responded more markedly to Na_2S and to KCNS than the other; but on subjecting this iron-charged residue to the action of 10 percent HCl solution for ten minutes, and then washing it in water,

the particles lost most of their contained iron. This suggests that the iron is an intrinsic component of the protein, rather than an impurity contributed either from the inorganic part of the tissue or from an external source, since in the latter case one would expect the iron to be more easily removed by treatment with HCl. This suggestion was strengthened by microscopic observations of effects, on enamel fragments, of KCNS in 2 percent HCl solution. As demineralization progressed, the enamel fragment remained colorless, but the protein fringe, at the periphery of the enamel fragment, assumed the red color.

It may be noted incidentally that the gray ash produced by simple incineration of enamel (prepared without contact with iron) yielded negative thiocyanate and ferrocyanide reactions, while the white (completely burned) ash produced by treatment with HNO_3 yielded positive reactions. Blank tests on the HNO_3 alone yielded very much weaker positive reactions.

Summary. These experiments indicate that enamel contains iron as an intrinsic component, apparently resident within or upon the keratin matrix. The physiological import of the iron has not been determined.

A METHOD FOR THE DETERMINATION OF IRON IN DENTAL ENAMEL

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Introduction. During a study of chemical properties of the keratin in dental enamel, Rosebury[2] obtained qualitative results indicating that this protein contains iron. The present paper records quantitative findings of a related preliminary study. Since the indicated proportion of iron in enamel is very minute, it was necessary to use a method that is applicable to the small amounts of iron expected. A satisfactory quantitative method must possess the desired sensitivity, and also use so few reagents that contamination with iron from this source can be excluded. A method that fulfills these requirements, and which further recommends itself by its simplicity, is that based on the reaction between α,α'-dipyridyl and ferrous ion, which produces an intensely colored complex that is stable over a wide range of pH. This method is well suited to a colorimetric determination of iron in various materials, since the number of interfering elements is small. The procedure finally adopted in this study, and described below, was a modification of the methods of Hill[3] and Müller,[4] adapted to dental enamel.

Experimental. The first step was the preparation of *iron-free reagents.* α,α'-Dipyridyl was prepared by Hill's modification of Blau's method. Iron-free sodium hydrosulfite was prepared by Hill's method: $Na_2S_2O_4 \cdot 2H_2O$ was dissolved in water at 40°C. to give a nearly saturated solution. Dipyridyl was added to maximum red coloration, the solution filtered rapidly, and the salt precipitated with ethyl alcohol. After twenty minutes, the product was filtered off, washed successively with 70 percent alcohol until colorless and with 97 percent alcohol, and then boiled up with 97 percent alcohol for ten minutes, filtered hot, and immediately dried in vacuo over

[1] William J. Gies Fellow in Biological Chemistry at Columbia University, 1933–34.
[2] Rosebury: *J. Den. Res.*, 1930, *10*, 187; 1934, *14*, 269.
[3] Hill: *Proc. Roy. Soc. London*, 1930, *107B*, 205.
[4] Müller: *Mikrochemie*, 1933, *12* (n.s. *6*), 307.

sulfuric acid. This product keeps well and gives no red color with either ferrous ion or dipyridyl. The other reagents were all of C.P. quality. Ordinary distilled water was used. All glassware was cleaned with chromic-acid mixture, and then rinsed carefully with tap-water and finally with distilled water. The vessels in which the analyses were carried out (small porcelain crucibles) were always handled with platinum-tipped forceps and kept covered with beakers.

The *colorimetric determination of iron* was carried out with a Zeiss Pulfrich step-photometer. In order that the calibration curve may be used, it is necessary to determine several points on the curve with solutions of known concentrations. This curve gives the relation between percentage transmission and extinction coefficient (1):

$$k_s = -\log \frac{i_s}{100} \tag{1}$$

where i_s is the percentage transmission, and k_s the extinction coefficient for a layer of solution s cm. in thickness. If k_1 is set equal to the extinction coefficient for a 1-cm. layer, then

$$k_1 = \frac{1}{s} k_s \tag{2}$$

When Beer's Law holds, the extinction coefficient of unit thickness of a solution of a colored substance is directly proportional to the concentration; therefore

$$c = Kk_1 = \frac{K}{s} k_s \tag{3}$$

where c is the concentration and k the proportionality constant. The standard solution was made up as follows: 0.8495 gram of $FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O$(M.W. = 392.14) was dissolved in water, 20 cc. of concentrated HCl added, and the solution diluted to 2 liters. This solution contained 0.06048 gram of Fe/lit., i.e., 60.48 γ/cc. A solution containing 10 γ Fe/cc. was prepared by diluting 16.53 cc. of stock solution to 100 cc. α,α'-Dipyridyl was used in a concentration of 1 percent in $N/10$ HCl, 1 cc. containing enough reagent to react with 3 mg. of Fe^{++}; 1.0 cc. of the dilute solution (10 γFe) was treated with about 5 mg. of Fe-free $Na_2S_2O_4 \cdot 2H_2O$ and 0.5 cc. of dipyridyl solution, and the mixture diluted to 10 cc. in a volumetric flask. As a control, 0.5 cc. of dipyridyl solution and about 5 mg. of sodium hydrosulfite were diluted to 10 cc. Both solutions were allowed to stand about fifteen minutes, for complete development of the color. The control was completely colorless when viewed in a layer 3 cm. thick. The solutions were then compared in the step-photometer, using Filter S50 (absorption maximum at 5100 Å.U.). The solution was set at 100:

$i_s = 44.5 \pm 0.2\%$ $k_s = 0.351$ $c = 10$ (γ in 10 cc.) $s = 3.0$ cm.

Using the calibration curve and equation (3), K = 85.8. A second determination, using 2.0 cc. of the iron solution, was carried out in the same manner. The control was colorless:

$i_s = 57.5 \pm 0.2$ $k_s = 0.238$ $s = 1.0$ $c = 20$ $K = 84.9$

The mean K is 85. The fact that the two determinations are in agreement within the limits of experimental error shows that Beer's Law holds even in very dilute solutions.

In this preliminary study,[5] which tested the applicability of the foregoing method, the *determinations were restricted to dental enamel*, which was prepared by Dr. Rosebury. "Teeth were cleaned in the usual way and desiccated. Enamel was prepared by crushing in pliers, the tooth surface being protected by two layers of clean rubber-dam and paper. The enamel fragments were ground thoroughly on all surfaces with new carborundum stones, and not washed or otherwise handled thereafter." (Personal communication from Dr. Rosebury.) Rosebury has shown that ignition of enamel with nitric acid yields a perfectly white ash. However, since ignition with HNO_3 leads to the formation of comparatively insoluble ferric oxide, it was necessary to dissolve the ash with concentrated HCl, evaporate to dryness, take up in dilute HCl, and add the dipyridyl and hydrosulfite. Three iron determinations in one preparation of enamel were made as follows:

(A) 113.4 mg. of enamel (dried over H_2SO_4) were weighed into a small porcelain crucible and treated with 0.5 cc. of concentrated HNO_3. The material was allowed to stand in contact with the acid for about ten minutes, when the acid was evaporated over a very small flame. Another 0.5-cc. portion of HNO_3 was added, the solution again evaporated to dryness, and the residue heated with a slightly larger flame for fifteen minutes. To the resultant cold perfectly white ash, 1.0 cc. of concentrated HCl was added and the solution taken to dryness on a steam bath. The residue, after trituration with 1 cc. of water and one drop of concentrated HCl, was heated on a steam bath to hasten solution, and then transferred to a 10-cc. volumetric flask with a dropper; the crucible was washed carefully with water, the washings transferred to the flask, and 0.5 cc. of dipyridyl solution added. Then $N/10$ NaOH was added until calcium phosphate just began to precipitate (ca. 2.0 cc.), and the precipitate was redissolved with one drop of $N/10$ HCl. This procedure brought the pH of the solution into the range in which maximum color is developed. About 10 mg.

[5] The study will be extended, in this laboratory, to determine the proportion of iron in enamel keratin, and the possible effects of induced anemia on the color and iron content of enamel of rat incisors.

of sodium hydrosulfite were then added to the solution, which was diluted to 10 cc. and allowed to stand about fifteen minutes to insure complete development of the color. This solution was compared with a control prepared in the same way, except that NaOH was not added since this would have made the solution much too alkaline. The enamel solution was well buffered by the phosphates. The solutions were compared in the step-photometer as in the case of the standard solutions:

$i_s = 81.0 \pm 0.8 \quad k_s = 0.086 \quad s = 5.0 \quad K = 85 \quad c = 1.46$ (γ in 10 cc.)

$$\textit{Percent of Fe} = \frac{1.46}{113400} \cdot 100 = 0.0013 \ (\pm 0.0004)$$

(B) Another determination was made in the same way: weight of enamel = 107.7 mg.:

$i_s = 96.6 \pm 0.4\% \quad k_s = 0.011 \quad s = 3.0 \quad c = 0.31$ (γ/10 cc.)

$$\textit{Percent of Fe} = \frac{0.31}{107700} \cdot 100 = 0.00029 \ (\pm 0.00004)$$

Mean percent of Fe (A and B) = *0.0008* (*±0.0002*)

The divergence of the results is due, in part, to the difficulty in obtaining homogeneous samples of enamel; in part, also, to the fact that the photometric readings were made in a range in which the instrument is least accurate.

(C) Another experiment was carried out in which 1.09 γ of Fe were added, in the form of ferrous ammonium sulfate in 1 cc., to 127.9 mg. of enamel and the mixture analyzed in the usual way. All of the added iron was recovered and the remainder was in good agreement with the results on enamel alone. Weight of enamel = 127.9 mg.; 1.09 γ Fe added:

$i_s = 70.5 \pm 0.6\% \quad k_s = 0.150 \quad s = 5.0 \quad c = 2.55$ (γ Fe in 10 cc.)

$2.55 - 1.09 = 1.46$ (γ Fe in 127.9 mg. enamel)

$$\textit{Percent of Fe} = \frac{1.46}{127900} \cdot 100 = 0.0011$$

Summary. A quantitative method for the colorimetric determination of minute amounts of iron in biological materials has been developed and described in detail. The method is based upon the reaction of Fe^{++} and α,α'-dipyridyl, which yields an intensely colored complex, the concentration of which may be determined conveniently in the step photometer. With this method it has been found that human dental enamel has an iron content ranging from 0.00029 to 0.0013 percent (mean, 0.0008). Iron added to enamel, and carried through this analytical procedure, may be recovered quantitatively.

The author expresses his sincere thanks to Dr. William J. Gies for suggesting this problem, and to Drs. Theodor Rosebury and Thomas B. Coolidge for supplying some of the materials used in the research.

RACIAL AND INDIVIDUAL VARIATION IN CERTAIN FACIO-DENTAL RELATIONSHIPS*

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Application of anthropometric techniques to problems of facial growth and development has resulted in formulation of "laws" and definite statements of dento-facial relationship. These have been accepted by some orthodontists as diagnostic of the degree of dental abnormality in a given instance. Not only are such measurements used in diagnosis, but they are accepted as criteria of development—as indicative of change in size and proportion. Utilization of the Frankfort Horizontal as a basis of orientation, both in growth studies and in studies of dental abnormality, raises the problem of reliability of at least its two terminal points; namely, *porion* and *orbitale*. Although the relative stability of these points gives them unquestioned value in the general orientation of the skull, one must never assume them to be stationary. They cannot be employed as fixed points, for their relation to each other and their positions within the complex pattern of the skull must vary with the course of growth and differentiation. Stability is one thing; stationary character is another. It is the purpose of this paper to analyze the variability of these points, both individual and racial, in the anthropometric measurements used in growth and orthodontic studies. In this analysis certain theories of cranio-facio-dental relationship will be considered. It is the essential purpose of this study, however, to evaluate relevant anthropometric measurements in terms of their variability. On this basis alone may we assess the reliability of either group or individual determinations.

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The cranial groups represented, and the number in each, follow[1] (all are male; 26 females were measured, but are not included):

White—Anglo-Saxon, 89; French, 33; Italian, 61.....	183	
Mongoloid—Tibetan, 32; Burmese, 35; Moriori, 46....	113	
Negro—Batatela, 59.....	59	355

The Anglo-Saxon, French, and Italian are presumed to be characteristic of the White race; the Tibetan, Burmese, and Moriori, characteristic of peoples having Mongoloid affinities; the Batatela, typical of the West African Negro. Each skull was placed in the Reserve Craniostat and oriented in the Frankfort Horizontal, i.e., the right and left poria (mid-point of upper rim of external auditory meatus) and left orbitale (lowest point, wherever found, of inferior orbital margin) were adjusted to a horizontal plane.[2] The relation of these points to one another, specifically the distance from porion to orbitale, must therefore be considered.

It is inherent in the nature of any craniostatic or craniographic device that employment of the Frankfort Horizontal almost automatically constitutes porion a central point. Cameron (3) has used it in his study of pre- and post-porionic dimensions. Hellman (7), Todd (13, 14), and Krogman (8) have used it in studying growing human, anthropoid, and mammalian skulls. Campion (4), Connolly (5), Harth (6), Oppenheim (9), Schwartz (10), Wallace (15), and others, have used it in the determination and analysis of dental relationships. Recently Broadbent (2) has demonstrated, by the use of the Broadbent-Bolton Roentgenographic Cephalometer, that the relative location of porion changes during growth. There seems to be no indication, however, that after termination of growth the porion changes its relative position. The fact remains, therefore, that the porion, because of its central position and its use as a landmark for the Frankfort Horizontal, serves as a point of departure in

[1] The material was studied while the writer was working in the laboratories of the Royal College of Surgeons of England as a National Research Council Fellow, 1930–31. To Sir Arthur Keith I record thanks for a cordial welcome and every assistance. To Miss M. L. Tildesley, research assistant in charge of human osteology, I am indebted for a stimulating interest in the present problem.

[2] For a precise definition of all anthropometric landmarks mentioned in this paper see *Biometrika*, Vol. XIV, pp. 196–200. For craniostatic technique, see Broadbent (1).

ascertaining relative positions of the several points used in dento-facial analysis. On this basis, therefore, I have located the positions of nasion and prosthion, and the level of the occlusal plane below the Frankfort Horizontal; have made the projected measurements of I2, C, Pm1, Pm2, M1, as seen from norma lateralis sinistra; and have studied the relation of facial measuring points to the dental arch. In all measurements employed, the number in the sample, range, mean, standard deviation, and coefficient of variation, together with probable errors, are given.

It must be specifically noted that the measurements recorded in this paper are *projected* measurements. By this I mean that distance between two points, or relation of these points to one another, has been determined by a craniostatic reading which treats the points as though they were in the same plane. For example, distance between porion and orbitale is measured as distance of orbitale from porionic axis; again, when tooth dimensions (antero-posterior) are stated, the projected length of the tooth is recorded by the craniostat from the buccal or lateral aspect. Actually, in practice, all measurements were transferred to millimeter paper. The method of projection has been employed so that the results will be comparable to the diagrammatic analyses made by those who have sought to demonstrate facio-dental relationships.

With the foregoing reservation in mind we shall turn to the first measurement, the relation of porion to orbitale (*table 1*). There is practically no racial difference in lengths of the porion-orbitale diameter, with the possible exception of Batatela in whom it is slightly less. Variability is fairly low, the coefficient ranging from 3.69 to 5.52. On this basis, in adult life, the porion-orbitale distance may be considered rather stable. The radius from porion as a center is an approximate measure of the forward projection of the face. Its variability, however, emphasizes that it can hardly be considered a constant, certainly not with reference to an individual analysis of cranio-facial proportion.

Nasion, namely the point where the median sagittal plane of the skull intersects the naso-frontal suture, has also been extensively used in facial measurements. We may consider, as an early example, the work of Welcker (16, 17) who, in studying growth in the human skull,

used porion as a center and measured growth by a series of radii to different points of face and jaw. Recently Krogman (8) has used the nasion parallel in his study of growth of the anthropoid skull. Let us see, now, how nasion is related to porion; first its distance from porion, measured along the Frankfort Horizontal, then its distance above the Horizontal (*table 2*). Again, no racial difference is to be observed, except for the Negro, in whom the distance is a bit less—a fact that

TABLE 1

Porion-orbitale distance

GROUP	NO.	RANGE	MEAN	S.D.	C.V.
Anglo-Saxon	89	69–92	78.13 ± 0.290	4.01 ± 0.202	5.12 ± 0.256
French	29	66–84	75.34 ± 0.424	3.40 ± 0.303	4.51 ± 0.397
Italian	61	71–85	77.18 ± 0.269	3.10 ± 0.188	4.02 ± 0.242
Tibetan	32	72–88	77.18 ± 0.438	3.69 ± 0.310	3.69 ± 0.404
Burmese	35	70–87	77.82 ± 0.444	3.89 ± 0.310	5.00 ± 0.404
Moriori	46	73–86	78.78 ± 0.297	2.96 ± 0.209	3.76 ± 0.263
Batatela	59	66–83	74.50 ± 0.364	4.11 ± 0.256	5.52 ± 0.343

TABLE 2

Porion-nasion distance

GROUP	NO.	RANGE	MEAN	S.D.	C.V.
Anglo-Saxon	74	75–99	84.51 ± 0.343	4.35 ± 0.242	5.15 ± 0.283
French	29	77–95	87.17 ± 0.444	3.58 ± 0.317	4.11 ± 0.364
Italian	60	81–95	88.27 ± 0.330	3.83 ± 0.236	4.34 ± 0.269
Tibetan	31	74–99	84.03 ± 0.539	4.47 ± 0.384	5.32 ± 0.458
Burmese	35	80–95	86.31 ± 0.397	3.51 ± 0.283	4.07 ± 0.263
Moriori	46	79–96	87.67 ± 0.350	3.54 ± 0.249	4.04 ± 0.283
Batatela	58	74–93	82.33 ± 0.384	4.34 ± 0.269	5.27 ± 0.330

may hark back to relative depression of the root of the nose, which obtains in this race. The measurement shows about the same degree of variability as that observed in the porion-orbitale dimension. Now let us observe the height of nasion above the Frankfort Horizontal (*table 3*). This measurement, which has little or no racial significance, is much more variable than the two preceding measurements, but its variability is probably not great enough to disqualify it as a basis of comparison.

Another point used extensively in anthropometric analysis of facial contour is prosthion, which is the intersection of the median sagittal plane of the skull with the alveolar margin between upper central incisor teeth. It is used in measurement of the degree of facial prognathism. The distance of prosthion from porion, measured along the Frankfort Horizontal, is shown in *table 4*. Variability of this distance is slightly less than that of the porion-orbitale radius, for

TABLE 3

Nasion above Frankfort Horizontal

GROUP	NO.	RANGE	MEAN	S.D.	C.V.
Anglo-Saxon	74	22–32	26.28 ± 0.141	1.79 ± 0.101	6.81 ± 0.377
French	29	27–33	30.45 ± 0.263	2.09 ± 0.182	6.86 ± 0.604
Italian	60	25–34	30.27 ± 0.215	2.47 ± 0.155	8.16 ± 0.499
Tibetan	32	25–34	29.75 ± 0.303	2.53 ± 0.215	8.50 ± 0.715
Burmese	35	24–34	29.23 ± 0.263	2.30 ± 0.182	7.87 ± 0.634
Moriori	45	27–37	31.89 ± 0.242	2.41 ± 0.168	7.56 ± 0.539
Batatela	59	21–36	27.71 ± 0.276	3.18 ± 0.195	11.48 ± 0.715

TABLE 4

Porion-prosthion distance

GROUP	NO.	RANGE	MEAN	S.D.	C.V.
French	30	84–98	90.87 ± 0.404	3.29 ± 0.283	3.62 ± 0.317
Italian	60	84–106	93.85 ± 0.418	4.81 ± 0.297	5.13 ± 0.317
Tibetan	32	85–102	92.66 ± 0.526	4.39 ± 0.370	4.74 ± 0.397
Burmese	35	86–104	94.71 ± 0.465	4.08 ± 0.330	4.31 ± 0.343
Moriori	41	91–105	96.76 ± 0.377	3.57 ± 0.263	3.69 ± 0.276
Batatela	56	82–104	93.23 ± 0.478	5.31 ± 0.337	5.31 ± 0.364

its C.V. ranges between 3.62 and 5.31. Its sole racial character is reflected in a contrast with the porion-nasion component, for then facial prognathism, i.e., forward advance of prosthion beyond nasion, can be measured. This leads to the observation that prognathism need not be forward projection of lower face; a shortened porion-nasion radius points more to backward rotation of upper face. In other words, porion-nasion projection distance is relatively shortened, so that porion-prosthion projection distance, a racial constant, becomes

relatively greater. This is borne out by measurement of the distance of prosthion below the level of the Frankfort Horizontal (*table 5*). The orbito-alveolar height, i.e., face below orbit, of the Negro is somewhat less.[3] The measurements are too variable for reliability, as indicated by a C.V. ranging from 5.99 to 10.61.

At this stage it will be well to take stock of our results. Stated succinctly, there seems to be no valid reason for rejecting measurements, in adult skulls, from porion as a center, on the basis of variability. Porion is vital in determination of the orientation of the skull; is central; and is literally the focus of cranio-facial measurements, especially in measuring protrusion of face from under brain-case. It must be emphasized, once more, that this applies to adult skulls only. Porion certainly shifts during growth, so that any attempt to base

TABLE 5

Prosthion below Frankfort Horizontal

GROUP	NO.	RANGE	MEAN	S.D.	C.V.
French	30	32–46	38.73 ± 0.505	4.11 ± 0.357	10.61 ± 0.927
Italian	60	32–47	38.35 ± 0.269	3.13 ± 0.195	8.16 ± 0.505
Tibetan	32	32–50	41.75 ± 0.512	4.29 ± 0.364	10.28 ± 0.865
Burmese	35	34–48	41.37 ± 0.283	2.48 ± 0.202	5.99 ± 0.485
Moriori	41	33–47	40.80 ± 0.330	3.13 ± 0.236	7.67 ± 0.573
Batatela	56	32–44	35.45 ± 0.249	2.77 ± 0.249	7.81 ± 0.499

craniometric analysis, in growth studies, on a "fixed point" will come to grief.

We must now consider nasion and prosthion in their relation to the Frankfort Horizontal. The first is quite reliable in facial measurements; the second, too variable to be dependable. Insofar as prosthion is closely identified with the tooth row, its variability becomes doubly significant. As concerns the relationship of porion, orbitale, and nasion, it may be concluded that orthodiagraphic tracings of adult skulls may, for comparison of detail, be superimposed upon Frankfort Horizontal, nasion-porion line, or nasion-parallel. The problem now

[3] In many male skulls, central incisors were knocked out at puberty. The resultant absorption affected the location of prosthion, but I have made an allowance for this based on skulls possessing these teeth.

before us concerns the relation of the dental portion of the face to these points or planes. Is there, for example, a fixed, "normal," relation between dental arch and orbitale? Knowing the position of one, can we predict the other—can we assess normality in terms of presumed constancy? The foremost exponent of constancy of facio-dental relationships is Simon (11),[4] whose "orbito-canine law" is based upon asserted dependency of orbitale and canine point: "Under normal conditions the orbital plane intersects the denture in the region of the canine; and it does this at any age—from five to forty years—in the temporary as well as the permanent teeth" (p. 71). This assertion has been challenged by several investigators, working with cranial material. Broadbent (1), Connolly (5), and Wolfson (18) have worked with adult skulls, while Hellman (7) has worked with the skulls of both children and adults. All of these investigators have reported that, on skulls, "Simon's law" does not hold, and that an orbito-canine relationship is far too variable to be of value in diagnosis. Simon stated his results for the German cephalic-type only, admitting and predicting racial variation. In accordance with this statement, I have tested the "law" on both cranial and cephalic relationships in other racial groups as hereinafter noted. Simon, himself, stated it might not "work" on other groups.

Any craniometric, cephalometric, or gnathostatic analysis of dento-facial relationship made from norma lateralis, and projected as a sagittal section from this norma, must be based on measurements as seen from a lateral plane. For example, on a skull looked at from the side the central incisors are hardly seen, the laterals only a little more, while the canine is usually the first tooth to offer its approximate full size to view. All measurements, then, must be made with the limitations of projection in mind. To this end I have measured first the level of the occlusal plane[5] below the Frankfort Plane, and then the width of I2, C, Pm1, Pm2, M1, as seen from the left side, and as registered on the Craniostat. Data for the level of occlusal plane below Frankfort Horizontal are given in *table 6*. Variability is uni-

[4] For a critical discussion of Simon's method see Tildesley (12).

[5] As here used "the level of the occlusal plane" is the value obtained by averaging distances, measured at right angles to the Frankfort Horizontal, of the occlusal surfaces of all teeth in the upper left-half of the jaw.

formly high, in keeping with the observed variability of prosthion below Frankfort Horizontal (*table 5*). The difference in actual measurement is apparently racial in origin, probably correlated with short- and long-faced racial groups. In *table 7* is presented the projected measurements of upper I2, C, Pm1, Pm2, M1, as seen from the left side.

These measurements, all extremely variable, served as the basis for an orthodiagraphic reconstruction, in each skull, of the level of the occlusal plane and projected dimensions of the several teeth, as seen from the left side. Porion, orbitale, nasion, and prosthion being likewise located, it was possible to note the relation of the orbital plane to the denture in each skull, to classify racial differences, and finally to

TABLE 6

Level of occlusal plane below Frankfort Horizontal

GROUP	NO.	RANGE	MEAN	S.D.	C.V.
Anglo-Saxon	87	41–57	49.68 ± 0.256	3.58 ± 0.182	7.21 ± 0.370
French	33	31–46	46.48 ± 0.438	3.75 ± 0.310	9.75 ± 0.811
Italian	54	30–47	47.25 ± 0.297	3.24 ± 0.209	8.26 ± 0.532
Tibetan	25	42–57	50.40 ± 0.472	3.51 ± 0.337	6.96 ± 0.661
Burmese	28	40–59	52.25 ± 0.593	4.65 ± 0.418	8.90 ± 0.804
Moriori	39	37–49	50.48 ± 0.370	3.41 ± 0.263	8.03 ± 0.611
Batatela	52	40–52	45.00 ± 0.303	3.26 ± 0.215	7.24 ± 0.478

measure the variability of this relationship in the entire series. The mode of intersection of the upper denture by the orbital plane, a perpendicular to the Frankfort Horizontal through orbitale, is defined below:

Anglo-Saxon, French, Italian, Burmese: between Pm1 and Pm2.

Tibetan: mid-point of Pm1.

Moriori: mid-point of Pm2.

Batatela: posterior Pm2.

In no group did the orbital plane regularly intersect the canine-point. The positions given above are modes based on the calculations contained in this paper. As an example (see also *figs. 1* and *4*) we may note frequency distribution of intersection for Anglo-Saxon skulls, on page 286.

TABLE 7

Projected measurements of I2, C, Pm1, Pm2, and M1; as seen from left side

GROUP	NO.	RANGE	MEAN	S.D.	C.V.
Width of lateral incisor					
Anglo-Saxon	64	3–6	4.26 ± 0.065	0.78 ± 0.046	18.24 ± 1.091
French	26	3–5	3.50 ± 0.080	0.63 ± 0.060	18.00 ± 1.701
Italian	56	3–5	3.68 ± 0.060	0.68 ± 0.040	18.48 ± 1.187
Tibetan	31	3–5	3.52 ± 0.067	0.56 ± 0.047	15.90 ± 1.372
Burmese	35	3–5	3.51 ± 0.067	0.60 ± 0.047	17.09 ± 1.386
Moriori	41	3–5	3.49 ± 0.053	0.54 ± 0.040	15.47 ± 1.160
Batatela	51	3–5	3.25 ± 0.053	0.55 ± 0.033	16.93 ± 1.139
Width of canine					
Anglo-Saxon	88	4–8	5.96 ± 0.060	0.83 ± 0.040	13.93 ± 0.708
French	29	3–7	5.37 ± 0.034	1.07 ± 0.094	19.93 ± 1.783
Italian	59	4–7	5.59 ± 0.074	0.85 ± 0.053	15.21 ± 0.948
Tibetan	32	4–7	5.56 ± 0.094	0.82 ± 0.067	14.75 ± 1.249
Burmese	35	4–7	5.60 ± 0.080	0.69 ± 0.053	12.32 ± 0.996
Moriori	46	4–7	5.26 ± 0.087	0.87 ± 0.060	16.54 ± 1.173
Batatela	57	3–7	5.21 ± 0.067	0.74 ± 0.047	14.20 ± 0.900
Width of first premolar					
Anglo-Saxon	88	4–8	6.01 ± 0.060	0.85 ± 0.040	14.14 ± 0.722
French	29	4–7	5.44 ± 0.101	0.81 ± 0.074	14.89 ± 1.324
Italian	60	4–7	5.68 ± 0.074	0.89 ± 0.053	15.67 ± 0.968
Tibetan	32	4–8	5.84 ± 0.094	0.79 ± 0.067	13.53 ± 1.146
Burmese	35	5–9	5.97 ± 0.107	0.94 ± 0.074	15.75 ± 1.276
Moriori	46	4–7	5.73 ± 0.080	0.79 ± 0.053	13.79 ± 0.975
Batatela	58	5–8	6.12 ± 0.074	0.81 ± 0.053	13.24 ± 0.831
Width of second premolar					
Anglo-Saxon	88	4–7	5.62 ± 0.053	0.74 ± 0.033	13.17 ± 0.675
French	29	5–7	5.83 ± 0.080	0.65 ± 0.060	11.15 ± 0.989
Italian	57	5–7	5.75 ± 0.060	0.65 ± 0.040	11.30 ± 0.715
Tibetan	31	5–7	5.97 ± 0.060	0.48 ± 0.040	8.04 ± 0.687
Burmese	35	5–7	5.91 ± 0.074	0.65 ± 0.053	11.17 ± 0.900
Moriori	46	5–8	6.00 ± 0.074	0.72 ± 0.053	12.00 ± 0.845
Batatela	59	5–8	6.07 ± 0.060	0.68 ± 0.040	11.20 ± 0.694
Width of first molar					
Anglo-Saxon	79	6–13	9.29 ± 0.101	1.31 ± 0.074	14.10 ± 0.783
French	27	7–10	9.04 ± 0.128	0.99 ± 0.087	10.95 ± 1.009
Italian	55	7–11	9.09 ± 0.087	0.94 ± 0.060	10.34 ± 0.668
Tibetan	31	7–11	9.32 ± 0.148	1.22 ± 0.101	13.09 ± 1.126
Burmese	34	8–11	9.65 ± 0.080	0.68 ± 0.053	7.05 ± 0.573
Moriori	45	6–11	9.24 ± 0.114	1.14 ± 0.080	12.39 ± 0.907
Batatela	59	6–11	9.75 ± 0.060	0.67 ± 0.040	6.87 ± 0.424

Canine: anterior	0	
mid	0	
posterior	4	
Between canine and first premolar	4	
First premolar: anterior	10	
mid	9	
posterior	14	
Between first and second premolars	11	
Second premolar: anterior	15	
mid	6	
posterior	9	
Between second premolar and first molar	2	
First molar: anterior	2	
mid	0	
posterior	0	86

The craniometric orbital plane is thus definitely unrelated to the denture insofar as constant intersection of canine is concerned. This may be demonstrated still further. In each skull I ascertained the distance from orbital plane to posterior margin of canine, or its alveolus (this measurement rather than canine tip was chosen because of wear or absence of the tooth). The related data are given in *table 8*. The extreme variability of this measurement, which is in substantial agreement with the coefficient of variation of 43.00 calculated by Broadbent, renders it absolutely unreliable as a constant in dento-facial diagnosis. It is obvious that the craniometric orbital plane in the adult skull rarely intersects the canine. Measurements of denture and dento-facial relationships, involving projection and reconstruction of proportions, are so variable as to prevent their formulation in any "law" of the "normal."

So far we have limited the discussion to craniometric measurements. Simon himself (p. 90) and Tildesley (12) are emphatic in declaring that the orbito-canine relationship, as stated, refers to cephalometric measurements. If, on the group of skulls studied, we are to obtain cephalometric measurements, we must make certain corrections (see Tildesley, 12): *First*, the cephalometric point is more medial[6] than

[6] Simon locates his cephalometric orbital point below the center of the pupil, when the subject is looking straight ahead. He states that the craniometric orbital point "lies at the lowest point of the orbital margin and consequently more to one side" (p. 90).

the craniometric, and hence may or may not be higher owing to downward slope toward the lateral orbital margin. *Second*, the cephalometric point will be more anterior—and hence farther from porion—than the craniometric owing to rounding of the lower orbital margin. This difference is enhanced when Simon, after palpating the orbital point, presses a vertical edge 1 cm. long against the lower orbital margin in line with the ascertained orbitale, which more often than

TABLE 8

Distance from craniometric orbital-plane to posterior margin of canine

GROUP	NO.	RANGE	MEAN	S.D.	C.V.
Anglo-Saxon	86	0–13	5.74 ± 0.236	3.29 ± 0.168	57.32 ± 2.974
French	29	1–11	4.79 ± 0.343	2.76 ± 0.242	57.62 ± 5.171
Italian	61	0–11	5.47 ± 0.256	2.96 ± 0.182	54.11 ± 3.343
Tibetan	32	0–10	3.00 ± 0.310	2.63 ± 0.222	87.67 ± 7.491
Burmese	35	0–11	5.94 ± 0.276	2.44 ± 0.195	41.08 ± 3.350
Moriori	46	0–13	7.45 ± 0.290	2.89 ± 0.202	38.79 ± 2.755
Batatela	58	3–16	9.91 ± 0.269	3.06 ± 0.188	30.88 ± 1.954

TABLE 9

Computed cephalometric orbital-point anterior to craniometric orbital-point

GROUP	NO.	RANGE	MEAN	S.D.	C.V.
Anglo-Saxon	89	7–14	10.00 ± 0.087	1.20 ± 0.060	12.00 ± 0.604
French	29	5–10	7.10 ± 0.168	1.32 ± 0.114	18.59 ± 1.659
Italian	61	5–10	7.03 ± 0.141	1.61 ± 0.106	22.90 ± 1.406
Tibetan	32	4–9	5.90 ± 0.168	1.42 ± 0.121	24.07 ± 2.050
Burmese	35	4–8	5.85 ± 0.121	1.07 ± 0.087	18.29 ± 1.488
Moriori	46	4–9	5.82 ± 0.107	1.11 ± 0.080	19.07 ± 1.351
Batatela	58	2–11	6.00 ± 0.134	1.51 ± 0.094	25.17 ± 1.591

not is inward on the rounded margin. *Third*, both foregoing differences will be increased by a more or less constant tissue thickness—skin and muscle (*Orbicularis oculi*). Sir Arthur Keith estimated this constant as 2 to 2.5 mm. By actual measurement[7] on 43 embalmed cadavera (Negro and White, male and female), I found the thickness to average 1.93 mm., with a range of 1.10 mm. to 2.75 mm.

[7] A sharp needle was thrust through the skin to the orbitale as palpated; the level of the surface of the skin was noted, and depth ascertained as the difference between needle-point and skin-level.

To a certain extent, to go from craniometric to cephalometric analysis of dento-facial relationships involves so many corrections and assumptions that accurate comparison is extremely difficult. If, however, we correct for the different location of orbitale, and for the possible effect of tissue thickness, the computed cephalometric orbital point will be located above and in front of the craniometric point. The data are summarized in *table 9*. The value for this correction is highest in Anglo-Saxon skulls; practically a constant in the other groups. In the first group, the lower margin of the orbit has a pronounced bony development, so that the vertical, 1 cm. long, pushed up against the orbit, is definitely anterior to orbitale palpated slightly in on the floor of the orbital aperture.

TABLE 10

Computed cephalometric orbital-point above craniometric orbital-point

GROUP	NO.	RANGE	MEAN	S.D.	C.V.
Anglo-Saxon	89	4–7	5.14 ± 0.040	0.59 ± 0.026	11.58 ± 0.587
French	30	2–6	4.10 ± 0.094	0.75 ± 0.067	18.29 ± 1.605
Italian	62	3–6	4.53 ± 0.080	0.91 ± 0.053	20.09 ± 1.221
Tibetan	32	2–6	3.59 ± 0.107	0.90 ± 0.074	25.07 ± 2.132
Burmese	35	2–5	3.20 ± 0.087	0.78 ± 0.060	24.38 ± 1.981
Moriori	46	2–5	3.60 ± 0.080	0.83 ± 0.060	23.06 ± 1.632
Batatela	58	2–5	3.72 ± 0.080	0.94 ± 0.060	25.27 ± 1.598

We may now consider distance of computed cephalometric orbital-point above craniometric orbital-point (*table 10*). Here, again, we encountered an extreme variation, of approximately the same degree as in *table 9*. Location of cephalometric orbitale, upon the basis of an ascertained craniometric orbitale, is extremely unreliable. If from the ascertained cephalometric orbital-point a plane is passed, perpendicular to the cephalometric Frankfort Horizontal, through the denture, we can re-assess the relation of orbital plane to teeth. The site of most frequent intersection of the cephalometric orbital-plane, in the several groups, is shown below (compare with page 284):

Anglo-Saxon: posterior I2[8].

French, Italian: anterior C.

[8] By "posterior" and "anterior" is meant posterior and anterior halves of the tooth.

Tibetan: between I2 and C.
Burmese: posterior C.
Moriori: between C and Pm1.
Batatela: mid-point of Pm1.

These locations represent modal positions based on all material. In no group did plane intersect canine with any degree of frequency—nor, indeed, did it intersect any one tooth with any constancy. As an example, we may note frequency distribution of intersection for Anglo-Saxon skulls (compare with page 286):

Lateral incisor: anterior	0	
mid	1	
posterior	4	
Between lateral incisor and canine	3	
Canine: anterior	7	
mid	9	
posterior	24	
Between canine and first premolar	11	
First premolar: anterior	10	
mid	11	
posterior	5	
Between first and second premolars	0	
Second premolar: anterior	1	
mid	0	
posterior	0	86

In order to demonstrate the relation of craniometric and cephalometric orbital-planes to each group studied, I have constructed a diagram to illustrate modal relationship in each, and here reproduce one for each racial stock: Anglo-Saxon, Burmese, and Batatela. In *fig. 1*, Anglo-Saxon skulls, the craniometric orbital-plane (*CR*) intersects the teeth between Pm1 and Pm2; the cephalometric (*CE*) is at the posterior half of I2. Average widths of teeth, position of prosthion (*PR*), and position of nasion (N), are also shown. In *fig. 2*, Burmese skulls, *CR* intersects between Pm1 and Pm2, while *CE* is at the posterior half of C. The average widths of teeth and positions of nasion and prosthion are shown. In *fig. 3*, Batatela skulls, craniometric orbital-plane is at the posterior half of Pm2; cephalometric orbital-

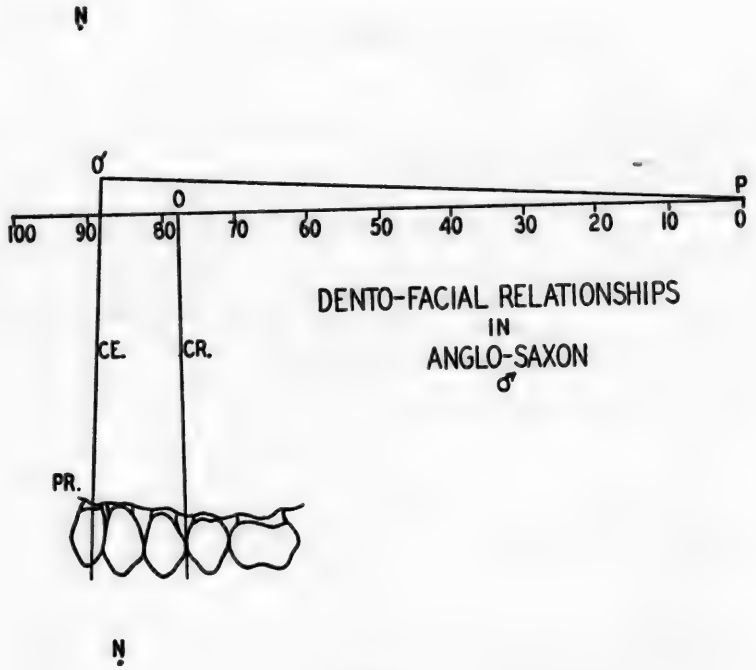
N
O'
P
0
100
90
80
70
60
50
40
30
20
10
0
DENTO-FACIAL RELATIONSHIPS
IN
ANGLO-SAXON
♂
CE.
CR.
PR.
N

FIG. 1

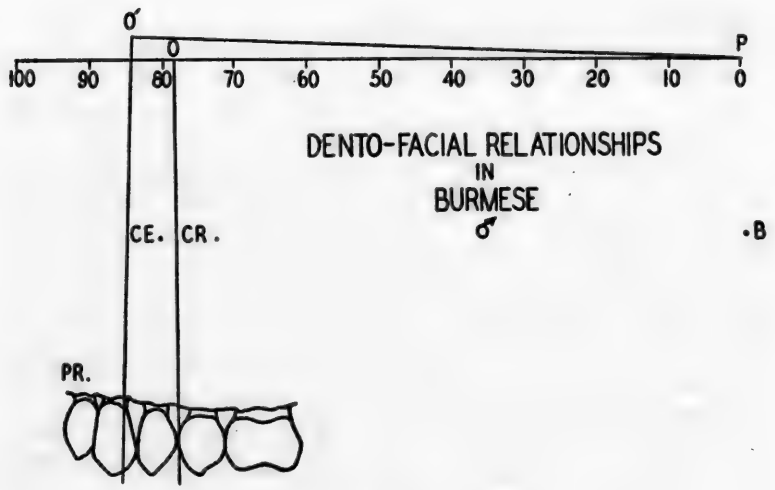
O'
P
0
100
90
80
70
60
50
40
30
20
10
0
DENTO-FACIAL RELATIONSHIPS
IN
BURMESE
♂
CE.
CR.
PR.
B

FIG. 2

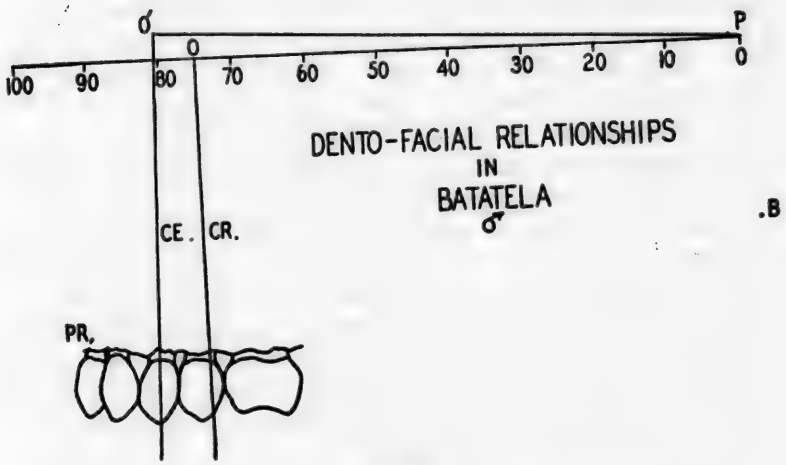
N
O
P
100
90
80
70
60
50
40
30
20
10
0
DENTO-FACIAL RELATIONSHIPS
IN
BATATELA
♂
.B
CE.
CR.
PR.

FIG. 3

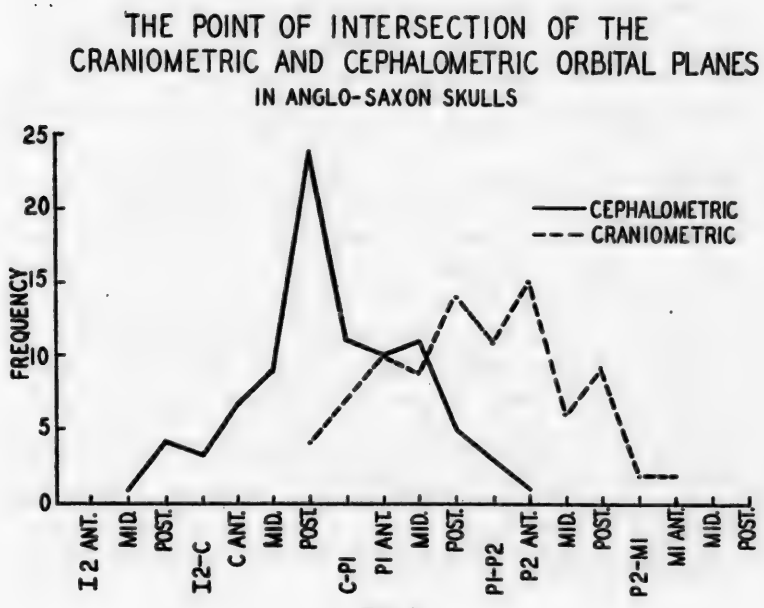
THE POINT OF INTERSECTION OF THE
CRANIOMETRIC AND CEPHALOMETRIC ORBITAL PLANES
IN ANGLO-SAXON SKULLS
FREQUENCY
25
20
15
10
5
0
CEPHALOMETRIC
CRANIOMETRIC
I2 ANT.
MID.
POST.
I2-C
C ANT.
MID.
POST.
C-PI
PI ANT.
MID.
POST.
PI-P2
P2 ANT.
MID.
POST.
P2-MI
MI ANT.
MID.
POST.

FIG. 4

plane is at the mid-point of Pm1. It is obvious that alveolar prognathism of this group has markedly affected the relationship of both planes. Average widths of teeth, and positions of nasion and prosthion, are shown.[9] *Fig. 4* presents an analysis, for one group—Anglo-Saxon—of range and frequency of site of intersection of the two orbital planes based on average dimensions. Cephalometric plane is definitely anterior to craniometric plane, but neither bears *constant, unvarying relationship to any tooth.* This is clearly shown by *fig. 4*, with the mode of the craniometric orbital-plane at posterior margin of canine, but with the range from mid I2 to anterior P2.

At this point it must be noted that at no time has the claim been made for the "orbito-canine law" that it is referable to any but German population. Perhaps this study works an injustice when it brings in the racial element; yet we cannot escape the conclusion that racial variants may well be reproduced as individual diagnostic problems. For example, the racial prognathism of the Negro may be found among certain non-Negro peoples. When faced with the problem of the prognathous *individual*, what will be the result? It is for this reason that an excursion has been made into racial differences. Facial prognathism, the extreme of which presents one type of malocclusion, has long been recognized as varying among races. In general, Negroids are prognathous; Caucasoids, orthognathous.[10] The measure of facial prognathism in the skull is the relation to the Frankfort Plane of two basilar diameters, basion[11] to nasion, and basion to prosthion, as expressed in the Gnathic Index: $\frac{\text{Basi-alveolar length} \times 100}{\text{Basi-nasal length}}$.

In order to test the reliability of basion as a locus of measurement, I erected a perpendicular to the Frankfort Horizontal through porion, and recorded the position of basion, either in front of, on, or behind this vertical plane; the level of basion below the Horizontal was also recorded. The two points thus serve to locate basion as a landmark. The data for the first measurement, given in *table 11*, are grouped so

[9] In these figures, P = porion; O = craniometric orbitale; O′ = cephalometric orbitale. In *figs. 2* and *3*, the position of basion (B) is shown.

[10] The values derived by the Gnathic Index are classified: x–98, orthognathous; 98–103, mesognathous; 103–x, prognathous.

[11] The point basion is determined where the median sagittal plane of the skull intersects the anterior margin of the foramen magnum.

that mean value represents distance of basion from porion without regard to sign, i.e., whether basion is in front of, or behind, the vertical through the porionic axis. If, however, all measurements are grouped on the basis of frequency in front of or behind the vertical, recording measurements in front as + and those behind as −, positional values are as follows:

Group	*No.*	*Range*	*Mean*
French	26	−3 to +6	+1.85
Tibetan	31	−6 to +5	+0.32
Burmese	35	−4 to +4	+0.74
Batatela	58	−6 to +3	−1.91

TABLE 11

Distance of basion from vertical projection of porionic axis

GROUP	NO.	RANGE	MEAN	S.D.	C.V.
French	26	0–6	2.31 ± 0.236	1.79 ± 0.168	77.49 ± 7.25
Tibetan	31	0–6	1.81 ± 0.188	1.54 ± 0.134	85.08 ± 7.29
Burmese	35	0–4	1.66 ± 0.148	1.29 ± 0.101	77.71 ± 6.27
Batatela	58	0–6	1.71 ± 0.148	1.71 ± 0.107	76.68 ± 4.80

TABLE 12

Position of basion below Frankfort Horizontal

GROUP	NO.	RANGE	MEAN	S.D.	C.V.
French	26	12–24	16.42 ± 0.458	3.48 ± 0.323	21.19 ± 2.002
Tibetan	31	12–25	17.77 ± 0.424	3.48 ± 0.297	19.58 ± 1.694
Burmese	35	13–28	19.34 ± 0.384	3.39 ± 0.276	17.53 ± 1.420
Batatela	58	12–25	19.79 ± 0.249	2.82 ± 0.175	14.25 ± 0.893

Mean value here indicates modal relationship of basion to porionic axis. Batatela, with a value of −1.91, indicating that basion is behind porionic vertical, is in contrast with the other groups. As for the location of basion *below* the level of the Frankfort Plane: the measurements in *table 12* do not express any racial difference. The outstanding result of the porion-basion relationship is its extreme variability, especially as seen in *table 11*. Obviously the two points are extremely unstable with reference to one another. Since we have seen that porion-orbitale and porion-nasion relationships are relatively

stable, it is probable that of the two points—porion and basion—the latter is the most variable.

This study may well be brought to a conclusion by comparison of the variability of measurements employed, grouped according to area and relationship. In each instance average variability, disregarding race, is given:

Measurements on face made directly from porion and Frankfort Horizontal

1. Porion to orbitale	4.52
2. Porion to nasion	4.61
3. Porion to prosthion	4.47
4. Nasion above Horizontal	8.81
5. Prosthion below Horizontal	8.42
6. Occlusal plane below Horizontal	8.05

Projected measurements of teeth as seen from left side

1. Lateral incisor	17.16
2. Canine	15.27
3. First premolar	14.43
4. Second premolar	11.15
5. First molar	10.68

Measurements relating cephalometric orbital plane to craniometric

1. Craniometric orbital plane to posterior margin of canine	52.49
2. Cephalometric orbital point anterior to craniometric	20.01
3. Cephalometric orbital point above craniometric	21.11

Measurements relating basion to Frankfort Horizontal and porion

1. Basion below Horizontal	18.14
2. Basion in front or behind porionic vertical	78.99

In the first group, only, do we find measurements with even a fair degree of reliability. In this group, length measurements made from porion as a center are the most reliable. Measurements of facial height are almost twice as variable. Measurements of tooth diameters, measurements purporting to reconstruct cephalometric orbitale from the craniometric, and measurements of a porion-basion relationship, are too variable for any degree of dependability. To a certain extent a spurious variability has been introduced into the measurements of level of nasion above F.H. and prosthion below F.H. The plane of the F.H., determined by right and left poria and by left

orbitale, has served as an arbitrary line of division. In reality, upper face height, from prosthion to nasion, has been divided into two parts at orbitale; variability has been correspondingly increased. Actually, the coefficient of variability of the distance Na-Pros is nearer 4.00 than 8.00. The same argument holds true for measurement of basion in front of, or behind, a vertical to F.H. through porion. The fact remains, however, that the method itself has an inherent variability.

Summary. 1. This paper is a study of variability of dento-facial measurements in 355 adult skulls of different races, in order to assess the reliability of certain presumed dento-facial relationships.

2. Variability of measurements of facial length in adult skulls from porion as a center, to orbitale, nasion, and prosthion, is relatively little. These dimensions may be accepted as reliable indications of forward growth of the face. Further, orthodiagraphic tracings may with confidence be superimposed upon each other in the Frankfort Horizontal, nasion-porion line, or nasion-parallel, to give comparability.

3. There is no dependable relationship between any facial point, or plane passing through that point, and any tooth in either the head (cephalometric orbitale) or skull (craniometric orbitale). Specifically, orbito-canine relationship is not constant, and orbital plane is extremely variable.

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INTRACELLULAR BACTERIA IN A DENTAL GRANULOMA

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The literature concerning dental infection and its relation to systemic disease has recently been reviewed by Appleton (1). Experimental work bearing on this problem has consisted, for the most part, of injection into laboratory animals of material from pulp or periapical tissues of infected teeth, either directly or after cultivation on artificial media. In dealing with periapical tissues it is difficult, if not impossible, to exclude the possibility of contamination with organisms from the oral cavity or from an infected root-canal. For this reason demonstration of the presence of intracellular organisms in a dental granuloma would be of interest. No report of the presence of such organisms in dental granulomata has been found in the literature. Nearly all investigators who have written on this subject speak of lack of phagocytosis of bacteria by inflammatory cells of dental granulomata.

Literature. Thoma (2) has demonstrated the presence of organisms of the actinomyces group in two dental granulomata. These organisms, found in routine examination of extracted teeth and attached tissue, had caused no symptoms of actinomycosis, and there was no recurrence of lesions after removal of the teeth and inflammatory tissue. Turner and Drew (3) stated that sections of dental granulomata have invariably shown the presence of organisms. Illustrations of organisms of coccus and diphtheroid types accompany their paper. No statement was made as to whether the bacteria were found in the capsule, or in the central portion of the inflammatory tissue. No evidence of cellular response to the presence of the organisms is gained from study of their photomicrographs. Such organisms might have been carried into the tissues during the processes of extracting

[1] Aided by a grant from the Milton Fund of Harvard University.

the tooth, or of preparing sections for microscopic examination. The same criticism applies to illustrations of bacteria in sections of dental granulomata accompanying a series of papers by Bulleid (4).

Rhodenburg and Franken (5) examined 100 granulomata by histological methods, and 65 by bacteriological methods. Eleven of those examined histologically showed acute inflammatory changes of a suppurative type, from which they deduced that organisms were present. They recorded no attempt to demonstrate bacteria in the inflammatory tissue. Harndt (6), after careful histo-bacteriological study of 50 dental granulomata, concluded that all solid granulomata are sterile. Where areas of degeneration were found, bacteria were frequently demonstrable in gaps and fissures of the tissue. He stated specifically that bacteria were never found in or among cells of the granulomata. He described distended cells, apparently similar to the foam cells to be mentioned in the present paper, but said that bacteria did not occur in their vicinity. He regarded the organisms, which he found in nearly all cases on the outer surface of the capsules of the granulomata, as contaminants introduced during the process of extraction. Weber and Pesch (7) reported findings for 119 granulomata. Bacteria were demonstrated in 11 percent of 100 granulomata examined by hfstological methods, and in 64 percent of 65 examined by bacteriological methods. These authors stated that, in spite of all precautions, single organisms from surrounding gum or mucous membrane may adhere to the outer surface of a granuloma. No illustrations accompany their article. In a histo-pathological study of the bacterial content of 28 dental granulomata, Kühn (8) reported that only 3 showed organisms which could not be excluded as contaminants introduced during extraction of the teeth. He referred to lack of phagocytosis of bacteria by the inflammatory cells. No illustrations accompany the paper, and no description of the location of the bacteria in the tissues is given. Feldman and Huttner (9) examined, by bacteriological and histological methods, 146 cases of "apical paradentitis." The fact that their cultures of the capsules were always positive makes it necessary to consider the possibility of contamination in each of their cases.

Material and methods. The dental granuloma to be described was encountered during routine examination of such tissue. The teeth,

after extraction, were dropped into 10 percent formalin solution, decalcified in 5 percent nitric acid, and embedded in celloidin, 200 teeth having been examined in this way. In 45, inflammatory tissue was attached to the apical portion of roots; in 18, this tissue, separated from the teeth after fixation in formalin solution to avoid decalcification, was washed for twenty-four hours in running water, refixed in Regaud's solution, embedded in paraffin, and stained to determine the presence of bacteria.

Case report. Mrs. B., age 34, in good health, married, one child. Lower right third molar had ached for one week; no roentgenographic examination; carious, but never filled nor root treated; pulp chamber and canal contained putrescent débris; extracted under novocaine anesthesia. Granuloma, approximately 3 mm. in diameter, attached to mesial root. Tissues preserved and prepared for examination as described above. Recovery after extraction uneventful. Roentgenograms three months later showed normal healing. *Histological examination.* Soft tissue imbedded in paraffin; sections cut at thickness of 2 to 4 microns. Wolbach's modification of Giemsa's stain, Gram's stain, and eosin methylene-blue stain used. Granuloma solid throughout; no fissures or foci of liquefaction. Outer border loose-textured, hyalinized connective-tissue, containing few scattered inflammatory cells, chiefly of lymphocytic series; occasional microörganism on surface. No cellular reaction to these organisms observed. Fairly numerous small blood-vessels penetrated capsule. Central portion of granuloma delicate network of connective-tissue fibers, among which were inflammatory cells of all types. Lymphocytes and plasma cells predominated; many large mononuclear phagocytes, chiefly of foam-cell variety, present; eosinophiles fairly abundant; occasional foci of polymorphonuclear leucocytes. This portion of granuloma contained numerous small thin-walled blood vessels; endothelial cells frequently swollen, and projected into lumen of vessel. Russell fuchsin-bodies, frequently found in dental granulomata, absent; strands of epithelium also lacking. Nearly all foam-cells in central portion contained bacteria, arranged about periphery of vacuoles in cytoplasm of cells, some containing few, others great many, bacteria. In these regions practically all organisms appeared to be within cells. Nearer capsule, some parts contained bacteria which seemed to be within cytoplasm of normal-appearing fibroblasts; other organisms in collagenous intercellular substance of inner portion of capsule. Morphology and staining reaction were similar in all three locations; gram-positive, not acid-fast, colored deep purple by Giemsa's stain, measured approximately 2 microns in length by

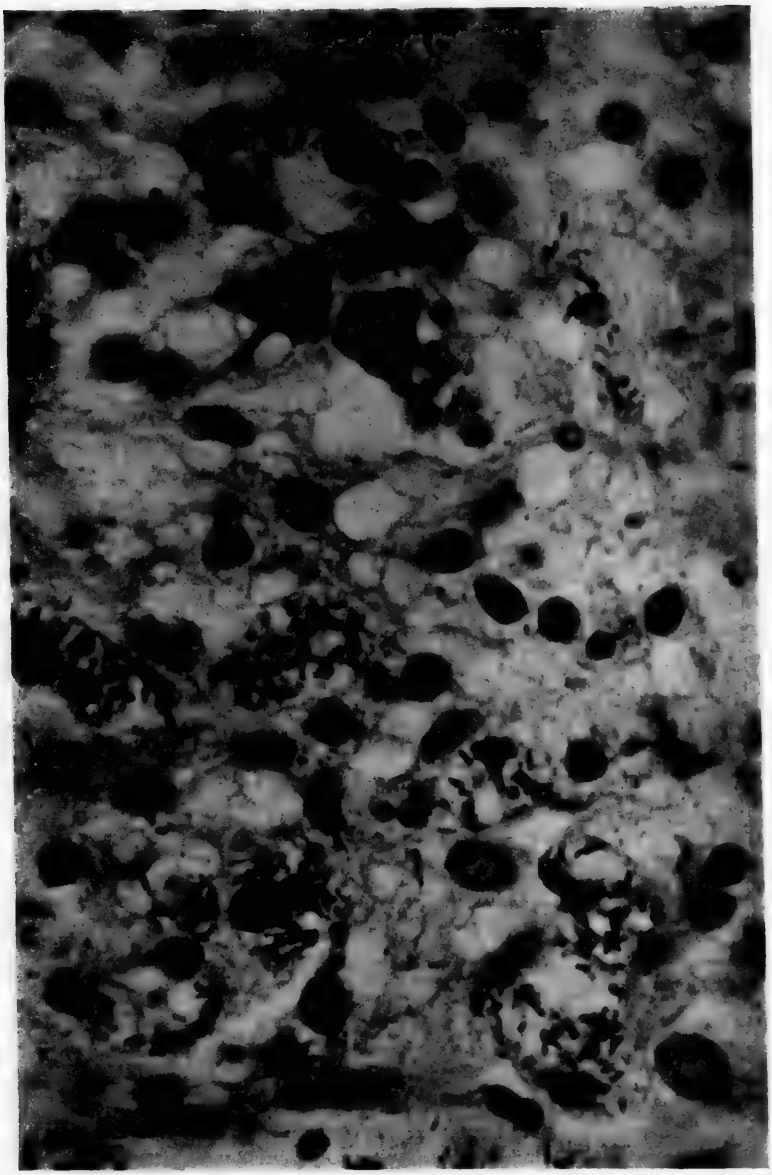

FIG. 1. Intracellular organisms in central portion of dental granuloma; Giemsa stain. × 1410.

0.6 micron in breadth; most were straight, some showed single or double curvature, ends rounded; very few, granular. Whether vacuolated bacteria-containing cells in central portion of granuloma are not degenerated fibroblasts rather than foam cells, is uncertain. They appear, however, to be similar in every respect, except for presence of organisms, to foam-cells commonly found in dental granulomata. Because of great numbers of bacteria within cells, compared to few definitely extracellular—and because little evidence is seen of degeneration of intracellular organisms—impression is gained that bacteria have multiplied within cells. (See *fig. 1.*)

Comment. With the exception of the present case, findings have been in accord with those of Harndt (6). Bacteria could be demonstrated regularly in the outer portion of the connective-tissue capsule of dental granulomata. No cellular reaction to these organisms has been observed, and they are regarded as contaminants. In no other case have organisms been seen within phagocytic cells. Bacteria were occasionally found in areas of necrosis in the centers of other granulomata. This dental granuloma differed from others only in the presence of intracellular bacteria. These findings prove conclusively that organisms were present in a dental granuloma of the solid type before extraction of the tooth. Unfortunately no cultures were made of the specimen. Gram-positive, non-acid fast, non-diphtheroid bacilli are rare; and in the absence of cultures, identification of the organism is impossible. An attempt is being made to secure cultures of the central portions of dental granulomata for correlation with histological examination.

Summary. Gram-positive bacilli have been demonstrated in large numbers within phagocytic cells in the central portion of a solid dental granuloma.

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COÖRDINATED REPORT OF THE COLUMBIA UNIVERSITY DENTAL CARIES RESEARCH GROUP[1]

FEBRUARY 1930–FEBRUARY 1934

FRANKLIN HOLLANDER, PH.D., *Secretary*[2]

Columbia University Dental Caries Research Committee

In February, 1930, there was initiated at Columbia University an extensive project for the investigation of the causes of dental caries. This project was made possible by the financial assistance of the Commonwealth Fund of New York. Because of the complexity of the problem, the research staff in charge of the work was organized into groups so that each of the senior members might be able to pursue that line of investigation which was dictated by his past experience. The project recently terminated its first period and has already started on a second under an extension of the original grant. It seems desirable at this time to coördinate the reports of the individual groups into a brief unified statement which will serve as a progress report of the committee as a whole. Since this report is not intended to be a review of the entire problem of dental caries, but merely an *ad interim* report of the activities of our own workers, it includes but little reference to the results of others. In the course of preparation of this summary it has become apparent that the results of the different groups tend in many ways to support each other. On the other hand, gaps and inconsistencies between different phases of the work have also been brought to light. Such an analysis effects a clarification of ideas preliminary to further work. As a brief summary of

[1] *Research Staff:* Edmund Applebaum, S. N. Blackberg, J. D. Berke, C. F. Bödecker (Chairman), H. W. C. Bödecker, L. R. Cahn, Franklin Hollander (Secretary, 1934–), Maxwell Karshan, Frances Krasnow, H. J. Leonard (Secretary, 1930–1934), E. C. McBeath, Theodor Rosebury, Richard Thompson. *Advisory Board:* H. T. Clarke, W. H. Eddy, F. P. Gay, A. F. Hess (deceased), J. W. Jobling, C. C. Lieb, Alfred Owre, A. M. Pappenheimer, H. C. Sherman, P. E. Smith, H. B. Williams.

[2] This report has been written by several members of the group in communication with all the others.

a large number of published articles, this statement contains none of the data which support its generalizations. To compensate for this deficiency there has been appended a bibliography of all the articles which constitute the original group reports. Those readers who desire detailed information on any specific point can obtain it by reference to the original contributions.

When this dental-caries research committee was first organized, two main hypotheses to account for susceptibility to dental caries presented themselves. The first of these regarded dental caries as a disease conditioned entirely by environmental factors—for example, bacteria, physical and chemical properties of foodstuffs which might adhere to teeth, and presence of areas on tooth surfaces in which fermentation could go on undisturbed. It was assumed that enamel is attacked from the outside by acid formed by action of lactobacillus organisms on carbohydrate food débris stagnating in protected pits and crevices about teeth. Incidence of dental caries, according to this hypothesis, was related solely to physical and chemical conditions (inciuding saliva) on tooth surfaces. Tooth enamel was regarded as a non-vital structure, and no allowance was made for metabolic conditions of the body which might influence the carious process on the enamel surface. The second hypothesis accepted also the fundamental notion of the first: that environmental factors play a rôle in the carious process. It differed from the first in emphasizing the possibility that physiological influences also may condition dental decay. There are two possible mechanisms through which such physiological influences might operate. On the one hand, there is the possibility that systemic influences operate by way of saliva; on the other, it is possible that they operate through the pulp and dentin. Conceivably, also, both these mechanisms might obtain. If systemic influences operate through the pulp, it is probable that dentin and enamel are permeated by a fluid derived from blood in the pulp (9). Through changes in this fluid, conditions in enamel might be modified so as to invite or resist the carious process.

It was the plan of our investigation to test these hypotheses by an attack along five lines: (a) histo-pathological studies of dentin and enamel, to gain a more complete picture of the structural changes associated with caries; (b) a comparative study of the chemistry of

saliva, blood, and teeth in individuals with and without caries, to determine whether any differences exist and, if so, what their significance may be; (c) a study of the bacteria found in carious and non-carious mouths, and of the manner in which they may operate to produce caries; (d) an effort to determine, in children, what nutritional factors, if any, produce the disease; and (e) experiments to produce caries in laboratory animals, and thus learn what factors are of importance in this process, and how the disease may be prevented by control of these factors. The data accumulated are for the most part consistent with the generalization that both environmental and physiological factors influence the carious process. It follows from this that the first hypothesis has been found incapable of explaining *all* the facts. Although it is possible, according to our animal experiments, for an extremely potent environmental factor to produce the disease in animals which are otherwise apparently normal, the evidence indicates that under ordinary human conditions factors other than the local environmental ones are also of great importance. The type of microörganism present in the mouth, the physical properties of the foodstuffs taken into the mouth, and the form and structure of the teeth, while important, do not alone account for the resistance of one individual and the susceptibility of another.

In man, we confirmed the observation that lactobacilli are more numerous in mouths with caries than in any caries-free mouths in which they are present (30, 36); also, we found that these organisms may be more resistant to acid in carious individuals (28, 30). Lactobacilli are known to be present always in carious mouths. We found that they are capable of producing a greater effective concentration of acid than other common bacteria (30). This faculty may make them the only organisms capable of dissolving enamel under conditions ordinarily obtaining in the mouth. This possibility is suggested by our finding that enamel appears to be about five times as soluble at pH 3.5, the level probably attainable by lactobacilli in the mouth, as it is at pH 4.5, which is not far from the limit for other bacteria under natural conditions (18). Lactobacilli are found frequently in caries-free human mouths (36, 30) and constantly in normal rat mouths (31, 32). In every case the organisms obtained from caries-free mouths were indistinguishable culturally or biochemically from those

present in mouths with caries (15, 29). The evidence therefore warrants the assumption that these organisms are essential to the dental-caries process, but mere presence of these organisms in a mouth is not alone sufficient to account for the production of dental decay.

In a series of experiments undertaken by one of our groups to produce dental caries in rats, various dietary conditions were tested (31, 32, 33). Some of these conditions were used because of their local effect on the teeth; among them were excess of fermentable sugar and use of several devices to make the diet adhesive in an effort to simulate the feeding of candy to children. No typical fissure-caries was produced in any of these early experiments. Nor was caries produced on diets in which the fluorine content had been greatly reduced (21, 24). It was not until a diet containing coarse grain-particles was used (34) that any success was attained in producing dental caries. When rice or corn was coarsely ground to pass through a 10-mesh screen, nearly all rats fed on it developed fissure-caries. This occurred whether or not the diet was nutritionally adequate, although decay progressed much more rapidly when the diet was deficient in minerals, vitamin D, and protein. If the grain was finely ground so that all passed through a 100-mesh screen, the diet otherwise remaining the same, no fissure-caries was produced. If the grain was cooked, no caries appeared. Fissure-caries was produced in these experiments by what appeared to be purely a local environmental effect. This was explained on the assumption that the coarse grain-particles wedge into pits in molar teeth where they remain for a considerable time and permit acid fermentation to occur freely. A very potent environmental influence was thus set up sufficient to produce dental caries in the laboratory rat, which ordinarily does not develop this disease. The experimental conditions here produced are probably not analogous to ordinary human conditions, since uncooked grain particles that tend to wedge in fissures in teeth very rarely occur in common human diets. Nevertheless, these results indicate that caries may on occasion be initiated through the agency of a highly potent external factor. In these rat studies only those lesions which could be recognized as beginning in the fissures were accepted as being comparable with dental caries in man. Other types of lesions, such as cavities beginning on the surface of the cusps or resulting from cusp fractures, were excluded from con-

sideration. Previous investigators of this subject have not made this distinction, a fact which should be considered in interpreting their findings.

The establishment of the fact that the rat is susceptible to dental caries, and the development of means for producing the disease at will, have effectively opened the field of experimental dental caries to more purposeful investigation than was possible before. Every judicious experiment in this field may now be expected to yield information of importance. Whether findings in rats will be applicable to the problem of human disease cannot be stated with assurance at present; but the striking similarity of the experimental lesions to those in young human beings warrants the working assumption that this is the case.

With regard to the second hypothesis, our results definitely indicate that resistance to dental caries involves one or more systemic factors, apparently related to calcium-phosphorus metabolism. Although it is possible under certain experimental conditions to cause carious cavities to form in the teeth of rats on certain fully adequate diets by means of a purely external agent, it has been found that the process of this decay can be influenced by dietary and other measures which affect the metabolic condition of the individual. Evidence for such influence, obtained both with experimental animals and with humans, follows.

The early experiments of one of our groups, in attempts to produce dental caries in teeth of rats by feeding diets defective in various nutritional factors such as calcium and vitamin D, were unsuccessful (31, 32, 33). Some of these experiments were carried through prenatal and postnatal life for a relatively long period (33). Marked structural defects occurred in teeth of animals on diets deficient in calcium and vitamin D, but no true dental caries was produced. On the contrary, experiments by another of our groups, using other diets imbalanced with respect to calcium, phosphorus, and vitamin D, have been successful in producing true fissure-caries in a high percentage of young rats (4). Diets used by both these groups contained no coarse grain-particles. An attempt to analyze and resolve this discrepancy is being made. In dogs, enamel lesions resembling human smooth-surface caries have been regularly produced on fully formed

teeth by feeding diets markedly imbalanced with respect to calcium, phosphorus, and vitamin D (5). In rats which developed fissure-caries on the coarse grain-particle diet, the time at which decay started and the rate at which it progressed were both markedly influenced by the adequacy of the diet (34, 35). In animals on diets deficient particularly with respect to calcium, phosphorus, vitamin D, and protein, the decay started more promptly and progressed more rapidly than in animals on an adequate diet. Experiments which involved feeding relatively large doses of viosterol or injecting parathormone were performed with rats on adequate diets (3, 6). It was found that both these procedures produced dental caries in a high percentage of animals. It follows from these experiments that systemic factors associated in some way with calcium-phosphorus metabolism are related to resistance of the teeth to decay.

Human dietary studies (25, 26, 27) were carried out on groups of children between the ages of eight and fourteen years, the various groups averaging about 25 children. Control groups at each of the institutions were maintained on the usual diets, which were of high nutritional standard. The diets for the study groups consisted of these basal rations supplemented by such items as were necessary to bring them up to the standards (slightly modified) for caries-prevention set by Boyd and Drain. The incidence of new carious cavities and the progress of old ones were carefully noted in all groups over a period of seven or eight months. In all cases, incidence of the disease, as measured by Bödecker's caries index (11, 12), was reduced significantly in those groups where additions had been made to the basal diet. Indeed, in 40 to 60 percent of the children comprising these test groups, caries was arrested entirely. This reduction in caries-incidence was consistently associated with diets containing more calcium, phosphorus, and the best sources of vitamin D than the controls. The study and control groups of one year were then interchanged the following year. The results were the same: incidence of caries in the new test-groups was reduced to almost zero, whereas the control groups, which had been nearly free from caries during the preceding year, now showed the usual incidence of caries among institutional children. It must be borne in mind that even on the regular institutional diets these children had, on the whole, a low caries-

incidence as compared with average children. Therefore, the effect of reducing the incidence of dental caries so much, by the dietary improvements introduced, is all the more striking. Previous to our work, other investigators had already shown that dietary considerations play an important part in the control of dental caries. However, the present contribution has attempted to evaluate several of the dietary factors involved. Our data in regard to changes in caries-incidence are made significant by reason of the size of the experimental groups, the duration of the periods of study, and the exercise of rigid supervision of the children.

Ultraviolet-light therapy proved much more efficacious as a protective agent against dental caries than other sources of vitamin D. This observation is similar to experience in the analogous fields of infantile and experimental rickets. Additions to the institutional diets of liberal amounts of the best known sources of vitamin C, in the form of oranges, orange juice, bananas, and tomato juice, had little effect on the incidence of dental caries. We have, therefore, no reason to think that possible deficiency of vitamin C in the American dietary is responsible for the great susceptibility of children to dental decay.

Although it is clear that metabolic processes exert a definite influence on susceptibility to dental caries, the details of the mechanism involved are not yet discernible from our biochemical data. No significant differences in pH or alkali reserve of the blood were found between carious and non-carious individuals, nor were significant differences detected in the blood levels of protein, calcium, and phosphorus between these groups (17). Furthermore, study of the distribution of individuals with and without caries among the four blood-groups gave no evidence for hereditary or innate susceptibility (37). The negative character of the studies so far performed in this connection does not rule out the blood as a vehicle for systemic disturbances, since other variables remain to be investigated. Similarly, our biochemical studies on saliva afford no evidence that this secretion is a vehicle for the action of physiological factors on the teeth. Investigations of the pH, titratable alkalinity, protein, calcium, and phosphorus of saliva from caries-free and caries-susceptible individuals have so far shown only insignificant differences (17, 20, 22, 23).

It was pointed out above that physiological influences might pos-

sibly operate through either or both of two ways—through the saliva or through a fluid ("dental lymph") derived from blood in the pulp. That changes in the pulp may be related to dental decay has been reported both in humans (14) and in experimental animals (7). For many years it was accepted that the enamel contained no organic matter and could not be permeated by fluid derived from the pulp chamber. But a continuous stroma of organic matter has been demonstrated (38) in human enamel by means of a histological technique devised for the purpose (8). Also, it has been shown by other workers that dyes injected into the blood stream of experimental animals may be transported throughout the dentin by way of the dental pulp, and removed again after the injections have been discontinued. This constitutes evidence for the normal penetration of lymph into the dentin, at least as far as the dentino-enamel junction. A microscopic tubular structure through which such penetration can occur has been described by one of our groups (13). There has also been advanced some evidence of a less conclusive nature to show that dyes can penetrate beyond the dentino-enamel junction into the enamel. Electro-endosmotic experiments on extracted teeth were carried out by one of our groups with both aniline dyes and mercuric chloride (1). It was found that the mercury could be driven into the enamel while, under the same conditions, the larger dye particles did not penetrate. This observation suggests that failure to obtain penetration of enamel by dyes in vivo may have been caused by the relatively large size of the dye particles used, and that at least the *inorganic* constituents of the lymph may ordinarily find access to the enamel. Further, histological examination of the teeth used in the electro-endosmotic experiments indicated that mercury penetrated the enamel along the organic enamel-rod sheaths.

Although a tissue fluid has never been isolated from dentin or enamel, the notion that such fluid may ordinarily be present is supported by the removal of water-soluble substances from these tissues. It has been demonstrated by one of our groups (19) that extracts of enamel and dentin contain small amounts of sodium, calcium, phosphorus, chlorine, and carbonate or bicarbonate. Small amounts of magnesium were also removed from dentin. Furthermore, a relatively large amount of protein was present in dentin extracts and a

detectable amount in enamel extracts.[3] The composition of a series of successive extracts showed that enamel and dentin contain material which is removed after five or six successive extractions. In addition, the slightly soluble apatite salts, which are said to constitute the major portion of enamel and dentin, pass into solution in constant amounts and alone persist in the seventh and subsequent extracts (as described). No differences in concentrations of these constituents (with the exception of protein[3] in enamel extracts for which comparison was not made) could be found in the extracts from carious and non-carious teeth. The quantitative relationships of these substances are such as to indicate that a small portion of the phosphorus and carbonate or bicarbonate in dentin and enamel is present as alkali salts.

Since the carious process appears to be related to calcium-phosphorus metabolism, we have attempted to find evidence of significant changes in density of calcification of teeth. Dogs with fully developed teeth were placed by one of our groups on diets deficient either in calcium or phosphorus and vitamin D, and their teeth were observed from time to time by x-ray examination (5). After a period of months, definite radiolucencies indicating demineralization of the dentin and enamel were detected, even though no outward sign of dental caries could be observed. Later, cavities having some of the characteristics of chronic smooth-surface caries occurred at certain favored spots on these teeth. X-ray studies of ground sections of human teeth also yielded evidence of a physiological variation in density of calcification. It was found that in dentin underlying a carious lesion in enamel there sometimes occurs an extensive area of increased radiopacity, which suggests the deposition of an excess of calcium salts (10). There is evidence, further, that density of calcification of human teeth is subject to change in the lifetime of the individual. Karlström, in Sweden, studied the hardness of enamel at various depths in teeth extracted from human subjects under a number of different conditions. He concluded that the inner portions of enamel normally increase in calcification with age. Furthermore, a period of mineral starvation,

[3] This statement is based on a positive reaction with the phenol reagent of Folin and Ciocalteu (*J. Biol. Chem.*, 1927, *73*, 627). The presence of phenols (tyrosin, etc.) was not excluded in these tests, and the detected phenol might not have been derived from protein. This point will be subjected to further investigation.

such as that which occurs during the later months of pregnancy in ill-nourished women, was reported to cause definite detectable decrease in hardness of enamel.

It was thought desirable to introduce into our work a tool which would lend itself to the study of density of calcification. The ordinary x-ray technique as applied either to whole teeth or to ground sections reveals few detailed variations in calcification other than gross differences between sound and carious enamel, or between dentin and enamel. A new radiographic technique (2) which utilizes soft x-rays, or Grenz-rays, has been applied to the study of ground sections of teeth. These soft rays are more easily absorbed than ordinary x-rays, and in general give radiographs showing much more detail. It has been ascertained by this method that the enamel of any one section shows organized variations in radiodensity which correspond to the so-called bands of Schreger (16). This observation suggests the possibility that the radiolucent bands constitute regions in enamel of relatively higher penetrability, which may play a rôle in the physiology of the tooth and therefore in the caries process. Other variations in radiodensity corresponding to already known structures in both dentin and enamel have also been observed by this method. The Grenz-ray technique is being applied, also, to a study of incipient caries, to analyze the progressive decalcification of the enamel which constitutes the first step in decay. Observations already made suggest that the decalcification of these areas occurs along definite channels rather than diffusely, as would be the case if the enamel were a solid of uniform porosity.

To conclude: these results obtained by our staff support the generalization that *the process of dental decay is associated both with environmental factors related to food retention and lactobacillus fermentation, and with physiological factors related to calcium-phosphorus metabolism.* To date, evidence as to the mechanism whereby physiological influences operate suggest that they work through the interior of teeth rather than through saliva. The second period of our project is concerned with attempts to elucidate further the mechanism of the carious process from these several approaches.

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ORAL OBSERVATIONS IN A CASE OF PERIODIC AGRANULOCYTOSIS[1]

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In 1922 Schultz (1) described a series of cases having an unusual blood picture, and accompanied by severe gangrenous conditions of mouth and throat. In all cases, onset was rapid and accompanied by chills, malaise, and fever. Blood examination showed red cells, hemoglobin, and platelets to be normal, but white cells were much reduced in number. Differential counts showed polymorphonuclear leukocytes either decreased or absent, while lymphocytes were relatively increased. Schultz used the term *agranulocytic angina* to designate such cases because of the association of granulocytic destruction with stomatitis. Meanwhile the literature on agranulocytosis in general has become abundant. However, to date apparently only two cases of periodic, or recurrent, agranulocytosis have been reported.

Brief reference to the case with which the present paper is concerned was made by Doan in 1932 (5). Previously, Rutledge, Hansen-Prüss, and Thayer (2) described the case of a male patient, 19 years old, observed from early infancy, regularity in occurrence of the "attacks" having been well authenticated. A representative differential blood-count (percent) during one of the periodic "attacks" was given, as follows: polymorphonuclear neutrophiles, 1.0; polymorphonuclear eosinophiles, 0.8; polymorphonuclear basophiles, 0.2; large lymphocytes, 32.2; small lymphocytes, 65.8. The red blood-cells and hemoglobin were within normal bounds, showing absence of anemia. Contrast the foregoing differential count (percent) with that of a normal condition, as follows: polymorphonuclear neutrophiles and eosinophiles, 70.0; polymorphonuclear basophiles, 1.0 (or less); large lymphocytes, 2.0; small lymphocytes, 25.0; transitional leukocytes, 2.0 to

[1] Read at the twelfth general meeting of the International Association for Dental Research, Chicago, Ill., Mar. 17 and 18, 1934; *J. Den. Res.*, 1934, *14*, 176.

10.0. We see that, during recurrent periods, which happened about every three weeks, there was marked leukopenia and relative lymphocytosis. To indicate reasons for dental interest in this type of case

FIG. 1

FIG. 2

FIG. 1. Patient's mouth, March 3, 1932; initial examination; gingival lesion, upper left first molar.

FIG. 2. Patient's tongue, March 18, 1932; lesions on tip accompanying an "attack"

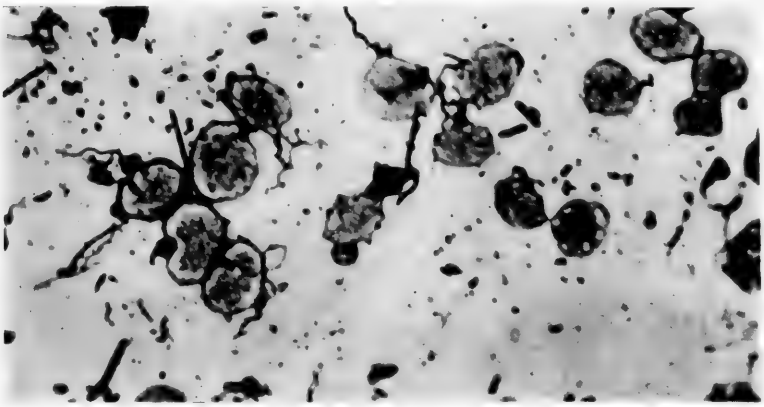

FIG. 3. Smear from gingival crevices, showing Vincent spirochetes: Class I

we quote from the summary of the article by Rutledge, Hansen-Prüss, and Thayer:

"The outstanding and commonest physical manifestations during an 'attack' are sore mouth, with swelling and reddening of the gums, with

shallow ulcerations and induration of the gums, lips, tongue and buccal membranes, associated with regional adenitis. From these sources Vincent's organisms have been obtained in large numbers. The only abnormal

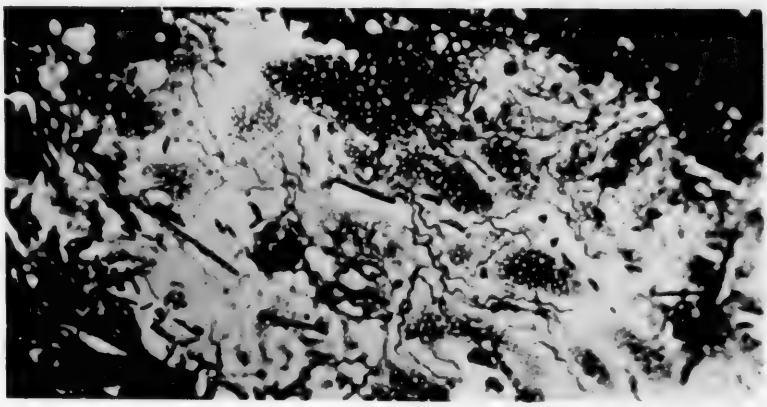

FIG. 4. Smear from gingival crevices, showing Vincent spirochetes: Class II

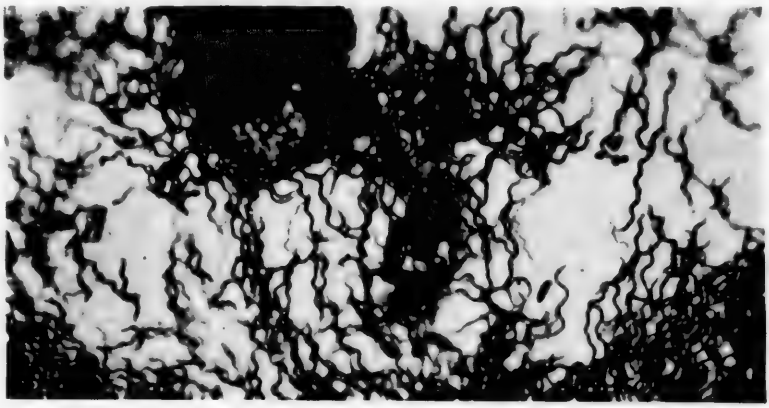

FIG. 5. Smear from gingival crevices, showing Vincent spirochetes: Class III

physical manifestation observed between 'attacks' is the chronic infection of the gums. Treatment of the infection of the mouth has resulted in the disappearance of the excess of Vincent's organisms in the intervals, but has not affected the periodic occurrence of fever and agranulocytosis nor the

stomatitis which occurs with them. During the height of the 'attack' these organisms are present in large numbers but diminish greatly in quantity toward the end of the 'attack.' "

Every effort was made to clean up the mouth in this case described by Rutledge, Hansen-Prüss, and Thayer, but, because of lack of coöperation from the patient, the effort was not a success. So much then for the only case of this type recorded in some detail in the literature.

It is quite conceivable that patients of this kind might apply first to dentists, for relief from stomatitis. A history of regular periodicity

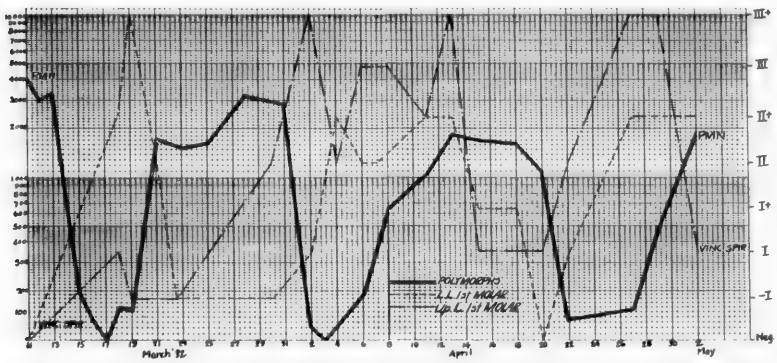

FIG. 6. Graph showing relation between Vincent spirochetes in gingival crevices and polymorphonuclear white-cells. Numerals at left edge indicate number of polymorphonuclear white-cells. Classes of smears (relative number of Vincent spirochetes) are indicated by numerals at right edge.

in any case should raise suspicions as to agranulocytic possibility. During the early part of 1932 our attention was called by Dr. C. A. Doan, of the Department of Medical and Surgical Research, to the fact that he had under observation a case of periodic agranulocytosis. He suggested that it be followed from the oral standpoint because of the relationship between mouth conditions and fluctuations in number of granular cells in the peripheral blood-stream, as pointed out by Rutledge, Hansen-Prüss, and Thayer.

Case history. Patient (P. D.); girl, about 18; first seen by us, *March 3, 1932.* Mouth indicated fair degree of care by patient. Lower left and right first bicuspids, and upper left first bicuspid, missing. Evidence of

lesion on buccal mucosa of lower left second bicuspid. Papilla between lower central incisors turgid. Badly swollen condition of marginal gingiva between lower lateral and cuspid. Thickened and turgid condition around buccal mucosa of lower right second bicuspid. Papilla gone between upper central incisors. Marked lesion on buccal gingiva at upper left first molar —most prominent gingival lesion; raised outer margin; apparent erosion of epithelium to knife-like edge at tooth surface, presenting circinate appearance shown in *fig. 1*, which also indicates general condition of mouth. Lesion on lingual of gingival margin at upper right first molar; similar condition on left. Gingivae on lingual of lower central incisors swollen and inflamed—later noted as constant condition. In general, however, condition of mouth showed chronic, rather than acute, inflammation. At this *initial* observation polymorphonuclear cells at high level (*fig. 6*); looking back, after many more observations on this mouth, abnormalities just noted were about as close to "normal" as tissues could go in interval between "attacks." *Mar. 18:* lesion appeared on tip of tongue (*fig. 2*): example of type of lesion accompanying "attacks;" sometimes occurred on tongue, sometimes on buccal mucosa; usually "sores" were more extensive, and quite painful. All marginal gingivae, and lesions mentioned in initial observation, now quite acutely inflamed. *Mar. 19:* no change. *Mar. 21:* tongue lesion healing. Lower anterior papillae turgid. Hemorrhage greater in buccal gingivae of upper and lower first molar areas, where smears were taken from gingival crevices. *Mar. 23:* tongue lesion gone. All gingivae more nearly normal. Hemorrhage in gingival crevices, where routine smears taken, much less than on Mar. 21. *Mar. 30:* no change. *Apr. 2:* no clinical observations. *Apr. 4:* all marginal gingivae hyperemic, especially on lingual of upper and lower incisors. Very small lesion on mucosa of left cheek opposite molar region. Lesion on buccal of upper left molar region quite marked. Soft palate yellowish. Complains of "sore gums." *Apr. 6:* lingual marginal gingivae of upper left molars red and swollen. Lingual marginal gingivae of lower incisors very hyperemic. Other marginal gingivae less inflamed. Buccal lesion on left cheek disappeared. Soft palate lost blanched appearance.

Above notes cover two normal and two "attack" periods. Clinical observations made over greater period, but detail would be mere repetition. Possible to predict, fairly closely, numerical condition of polymorphonuclear cells in blood stream from examination of mouth.

During three complete cycles of this case, while oral observations were being made synchronously with differential blood-counts, smears were made at each observation from the following gingival crevices:

(1) lower left first molar; (2) upper left first molar; (3) lower left central incisor. In each smear a complete excursion was made through the buccal part of the crevice. Smears were mordanted with 1 percent potassium permanganate for eight minutes; then stained with 2 percent methyl violet for a similar period. This method stains all spirochetes very strongly. The entire smear was examined for Vincent spirochetes. While no quantitative method was employed, all examinations were made by one individual and smears classified, on the basis of occurrence of Vincent spirochetes, into a series which, starting from a negative condition in that regard, advanced through seven arbitrary classes (*fig. 6*) to what was designated as a Class-III-plus occurrence. Main classes, designated I, II, and III, are shown in *figs. 3, 4* and *5*.

With no previous knowledge of differential blood-counts during this part of the period of observation, findings from smears of upper and lower-molar crevices (lower-incisor crevice-smears agreed with the two illustrated) were plotted on semi-logarithmic cross-section paper, and the differential count for the same period was added to the graph (*fig. 6*). We believe that this graph indicates marked relationship between number of granular cells in the peripheral blood-stream and activity of Vincent spirochetes in gingival crevices. With the single exception of marked rise in number of spirochetes in the crevice of the upper left first molar during the period from March 22–31, there is definite inverse relationship between polymorphonuclear-cell number and Vincent-spirochete number. Furthermore, variation in spirochete condition follows periodicity of polymorphonuclear cells, with a definite lag indicating that the spirochete condition results from lowering of the granular-cell count. For this reason we are not inclined to view Vincent infection as in any way a cause of the lowering in granular-cell number. Rather it may be stated that, all other factors being equal, polymorphonuclear cells in the peripheral blood-stream tend to inhibit increase of Vincent spirochetes in gingival crevices. In this connection, the following comment by Kastlin (3), on a microscopic examination of mouth ulcers, at autopsy, of a case of agranulocytic angina is of interest: "Beyond the necrosis there was no inflammatory-cell reaction excepting an occasional lymphocyte, endothelial and plasma cell. These cells formed no particular zones of reaction. There were no polymorphonuclear leukocytes." While no biopsy

material could be obtained from our case, it would be reasonable to suppose that the condition described by Kastlin obtained in tissues of this patient during "attack" periods; and that promptly, upon the appearance of granular cells, the lesion caused by spirochetal toxins began to heal and the spirochetes themselves (not in the tissue but outside of it in gingival crevices) were affected, as shown by reduction in number.

Our case differed from the one reported by Rutledge, Hansen-Prüss, and Thayer in that the patient cared for her mouth in a manner above average. Even with this care, however, the period of high polymorphonuclear-count was not long enough for complete repair of damaged mucous tissues. We made no attempt to scale teeth, or to medicate gingival crevices, because we wished to interfere in no way with the oral sequence of events and their relation to general conditions. This mouth will recover, and stay recovered, only when some way is found to overcome the periodic fall in granular-cell count. To date this case has been followed by the Department of Medical and Surgical Research for three years and five months, but all attempts to control periodic fluctuations of granulocytes have failed. These attempts have included liver extract, nucleotide injections, and splenectomy. The case will soon be reported, in detail, by Dr. Doan.

Summary. There is presented a description of oral conditions in a case of recurrent agranulocytosis. To the best of our knowledge this is the second case of this type to be reported in the literature. The only case previously described is briefly reviewed, with special attention to oral manifestations.

Our oral observations confirm findings of Rutledge, Hansen-Prüss, and Thayer concerning the massing of Vincent spirochetes at times of low granulocyte-count, present findings being confined to gingival-crevice conditions. Increased clinical signs of inflammation of gingivae, and buccal and lingual mucosa, accompanied increase in number of Vincent spirochetes.

By plotting fluctuations of granular-cell number and spirochete condition, together, it has been shown that increase in Vincent spirochetes followed lowering of granular-cell count, thus indicating that number of polymorphonuclear cells in the peripheral blood-stream is an important factor in the control of growth of Vincent spirochetes

in gingival crevices, and of effects on the mucosa consequent to such growth.

Cases of this type, in varying degrees of severity, may be much more numerous than has been assumed. It is natural that such patients should apply first to the practitioner of dentistry. With a knowledge of relationship between blood condition and oral mucosa, as definitely demonstrated in this case and the one reviewed, dentists will have a better conception of the oral problem involved, and also will serve the welfare of their patients by suggesting studies of the blood picture by competent physicians.

We wish to thank Dr. C. A. Doan for his helpful suggestions; for the opportunity to follow this case; and for the use of data on the differential blood-count.

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INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Organized: New York City, Dec. 10, 1920. Of those at the meeting of organization, the following remain active members: Adolph Berger, Theodor Blum, G. W. Clapp, H. S. Dunning, W. J. Gies, H. W. Gillett, R. G. Hutchinson, Jr., T. P. Hyatt, Milo Hellman, F. C. Kemple, A. H. Merritt, B. B. Palmer, P. R. Stillman, L. M. Waugh.

General Meetings have been held on this schedule: (1) New York, Dec. 21, '22. (2) Chicago, Mar. 7, '24. (3) Chicago, Mar. 20, '25. (4) Chicago, Mar. 26, '26. (5) New York, Apr. 19, '27. (6) Washington, Mar. 25–28, '28. (7) Chicago, Mar. 23–24, '29. (8) Toronto, Mar. 24–26, '30. (9) Memphis, Mar. 21–22, '31. (10) Columbus, Mar. 19–20, '32. (11) Chicago, Mar. 18–19, '33; adjourned executive session, New York, May 25, '33. (12) Chicago, Mar. 17–18, '34. *Next general meeting* (13): Chicago, Mar. 16–17, in coördination with the twelfth annual meeting of the American Association of Dental Schools, Mar. 18–20, 1935.

Objects (*Const., Art. II*): . . . "(a) To promote broadly the advancement of active research in all branches of dental science and in related phases of the sciences that contribute directly to the development of dentistry, and which add to the knowledge of the mouth and teeth and of their relations to the body as a whole; . . . (b) to encourage and facilitate coöperative effort and achievement by, and mutual helpfulness among, investigators in all nations in every division of stomatology; (c) to the end, particularly, that dentistry may render cumulatively more perfect service to humanity." (The Association has been using "dentistry" and "stomatology" as synonyms.)

Membership: total (June 15), 360. *Eligibility* (*Const., Art. 14, Sec. 1*).—"Any person who has conducted, and published an account of, a meritorious original investigation in dental science, or in any of the sciences contributory to stomatology, shall be eligible to membership in this Association, providing such person conforms to the recognized standards of professional ethics." *Election.*—New members may be elected *only at general meetings of the Association.* Membership in a section is secondary to membership in the Association as a whole. Removal of members from one geographic center to another does not affect their membership in the Association.

Endowment fund (as of April 21, 1934): $1,119.23. There have been no expenditures from this fund.

Divisions may be organized by the members in any nation. There are now divisions in Austria, Canada, China, Czechoslovakia, England, Hungary, South Africa, and the United States—8. *Sections* may be organized by the members in geographic centers within the divisions. There are now sections (26) in Vienna ('29); Halifax ('28), Toronto ('21), Winnipeg ('30); Chengtu ('34); Prague ('32); London ('31); Budapest ('34); Johannesburg ('34); Ann Arbor ('23), Baltimore ('33), Boston ('20), Chicago ('20), Cleveland ('30), Columbus ('32), Louisville ('32), Minnesota ('28), New Haven ('30), New York ('20), Philadelphia ('28), Pittsburgh ('28), Richmond ('33), Rochester ('33), St. Louis ('28), San Francisco ('24), Washington, D. C. ('31). The names of the secretaries are given on p. 2 of this cover.

Sectional meetings in 1933–34 (*schedule subject to revision*). *Ann Arbor*—May ?. *Baltimore*—Oct. 24, Nov. 15, Jan. 31. *Boston*—Nov. 6, Jan. 29, May 21. *Chicago*—Oct. 27, Jan. 26, May 18. *Cleveland*—Feb. 12. *Columbus*—Mar. 9. *Halifax*—Nov. 19, Jan. 25, Mar. 14. *London*—Oct. 18, Dec. 6, Jan. 24, Apr. 18. *Louisville*—May 11. *Minnesota*—Nov. 20, Jan. 22, Feb. 26. *New York*—Dec. 7, June 7. *Philadelphia*—Oct. 13. *Pittsburgh*—Oct. 2, Nov. 6, Dec. 4, Jan. 8, Mar. 19, May 7. *Prague*—Jan. 12, May ?. *Richmond*—Nov. 18, Apr. 18, May 26. *Rochester*— Oct. 13, 27; Nov. 10, 24; Dec. 8, 22; Jan. 5, 19; Feb. 2, 16; Mar. 2, 16; Apr. 13, 27; May 11, 25. *St. Louis*—Nov. 14, Jan. 9, Apr. 10. *San Francisco*—Nov. 16, Jan. 24, Feb. 28, Mar. 28, Apr. 25. *Toronto*—July 17, Sept. 12, Oct. 20, Jan. 18. *Washington*—May 3. *Winnipeg*—Feb. 7, May 28.

Journal of Dental Research: now the Association's official publication. See register of officers and editors on page 2 of this cover.

JOURNAL OF DENTAL RESEARCH

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Quotation from a letter to the honorary editors by the retiring management.—"We have endeavored to make the *Journal of Dental Research*, since its establishment in 1919, an exemplar of the best and most meritorious in non-proprietary publication of dental research. The honorary editors have contributed the strength of their approval and support of that effort, and have responded to many calls for assistance. We cordially thank you for the very valuable aid you have given. Your interest and sympathy have fortified the *Journal's* determination to attain its avowed objectives. Your cooperation, accredited in each issue as a public endorsement of our purpose, has facilitated the *Journal's* growth and enhanced its usefulness. The *Journal*, on your recommendation, is about to pass into the complete control of the International Association for Dental Research, which will receive it as a gift from the original management. The Council has voted to place the *Journal* under the direction of a board of editors representative of, and to be selected by, the sections of the Association. Each of you, as a member of the Association, will have opportunity to participate in the recurrent selection of these editors. It is deeply gratifying to us, as we formally sever our past relationship, that the *Journal*, as the official publication of the Association, will continue to have the abiding interest and individual support of its former honorary editors."—*William J. Gies, Executive Officer, Board of Editors.*

INFORMATION FOR PROSPECTIVE SUBSCRIBERS OR CONTRIBUTORS

THE JOURNAL OF DENTAL RESEARCH is dedicated to the advancement of research, and to the dissemination of knowledge, pertaining to the teeth and mouth and to their relations to the body as a whole. It publishes original investigations and reviews in these fields, and in the sciences allied with or applied to them. Although without a "policy," the JOURNAL represents an unwavering purpose to keep widely open the channel to freedom of *responsible* and *respectable* expression of truth and opinion, by individuals or organizations, without qualification by or for any views its editors individually may entertain. Issued bi-monthly, at the *ends* of the months of February, April, June, August, October and December, each volume contains at least 500 pages, for which the subscription price is $5.00.

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